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Zebrafish in the Wild: Microhabitat Use by Zebrafish *Danio rerio* (Hamilton, 1822) from Karala River of Jalpaiguri District, Northern Bengal, India

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Abstract

The microhabitat preference of zebrafish, *Danio rerio* from Karala river was investigated. The availability/preference of microhabitat variables such as (i) distance from the stream bank (ii) water column depth (iii) mesohabitat (iv) water velocity (v) substratum and (vi) subaquatic vegetal cover were quantified based on underwater observations covering a total of 400 m^2 of stream area. Microhabitat selectivity was analyzed by comparing the microhabitat availability in the study site and the microhabitat used by fish. Data availability and microhabitats usage pattern were used for calculating habitat availability/preference of *D. rerio*. In order to test the microhabitat preference of *D. rerio* the Principal Component Analysis (PCA) and Canonical Correspondence Analysis (CCA) were applied. In PCA the first three components with higher Eigenvalue accounted for about 98.46% of the total variance. The first component axis alone explained 86.33% of the variance with an Eigenvalue of 5.3 with high loadings (>0.7). CCA revealed a clear separation of the *D. rerio* along the microhabitat variables. Among the thirty two microhabitat variables, the forward selection procedure was found to be positively significant with eighteen microhabitat variables P<0.05. The preference of *D. rerio* in the studied streams *viz:* pool, plunge pool and isolated pool; slow flow, moderate flow and fast flow; and the substratum parameters: sand, gravel and leaf litter; depth parameters like Depth 1, Depth 2, Depth 3 and Depth 5; fish cover parameters such as root undercut and distance from bank parameters such as Distance from bank 1, Distance from bank 2 and Distance from bank 3. CCA revealed a clear distribution pattern of the *D. rerio* along the microhabitat variables.

Keywords: *Zebrafish*; Snorkeling; Microhabitat; Non-stochastic use; Preference

Introduction

The nature of habitat has an important bearing on the distribution, abundance, growth and other characteristics of fish populations [1,2]. The physical nature of a stream determines the quality and quantity of habitat formation available to the distribution of organisms in addition to the aesthetic and amenity values [3]. The co-existence of many ecologically similar, closely related fishes on the same habitat continues to be a topic of considerable debate in stream ecology [4]. Many species have been adapted to the natural flow regime [5,6] and the temporal dynamics of habitat quantity may be a major determining factor towards the fish population responses in riverine environment [7]. Over the past three decades much research work has been carried out on environmental flows though the research on habitat use *vis-a-vis* changing flows remains elusive [8]. Only limited evidences are available on the different patterns of habitat use and large numbers of empirical case studies were unable to develop general relationships [9].

The habitat attributes include physical features, water quality and biological components [10]. Data on habitat use provides a base for predicting the population response and the specific abundance in specific habitat type [11]. Different populations and communities use different habitat types, delineation of these patterns could provide a base to predict the biotic responses in relation to the habitat changes. Previous reports are available on the habitat relationship in streams [12-14] with important insights. This approach of habitat specific estimate of abundance can be combined to predict the population abundance in the stream segment as a whole [15,16] slow versus fast riffles from which fish species requirements/usage can be developed. Stream biologists have also addressed the lateral microhabitat dimension based on main channel to hydrologically isolated shallow habitats [17,18]. Stream habitat components identification is important to understand the ecological relationship between habitats and biota, to assess the environmental changes and to provide options for stream management [19,20].

The zebrafish, *D. rerio* is an omnivorous freshwater fish native to the rivers, streams and rice paddies of east India, Bangladesh and Burma [21,22]. Zebrafish have typically been described as inhabiting on slow-moving or standing water bodies, along the edges of streams and ditches, in particular adjacent to rice-fields [23-25]. However, they are also reported to inhabit rivers and hill streams [26]. And nowadays, it is being a popular model system for developmental biology and genetics [27-29].

The habitat preference of the zebrafish has been systematically described based on the following 4 surveys in India and Bangladesh. McClure [22] captured zebrafish in three sites in the Ganges drainage. Spence [30] captured in nine sites in the Ganges and Brahmaputra drainages. Engeszer [31] recorded in fourteen sites in the Ganges and Brahmaputra drainages. Arunachalam [32] captured in 21 wild populations from streams/rivers of the Western Ghats, Western and North-Eastern Himalayas. In all four studies, zebrafish were found to occur in shallow waterbodies with a visibility at a depth of ~30 cm, frequently in unshaded locations with aquatic vegetation and silty substrate.

In nature, zebrafish are often associated with aquatic vegetation, feed at the surface and from the substrate of zooplankton and insects, phytoplankton, filamentous algae and vascular plant material, spores and invertebrate eggs, fish scales, arachnids, detritus, sand and mud have been reported from their gut contents [32,33]. Recent studies have begun to examine questions in ecology and evolution using natural populations of zebrafish, which occur in India, Nepal and Bangladesh [31,34]. These include: behavioral genetics of shoaling activity level, boldness and aggression [35], feeding ecology [22], reproductive behavior [36], colour pattern variation [37], genetic effects of domestication [38,39], variation in individual growth rates [40] and the number of recessive lethals in wild-caught populations [41].

Only very little information is available on the natural behavior or biology of zebrafish and few studies have been conducted on wild populations [31-33,42-45]. However, some of the work that has been undertaken suggested the conditions under which zebrafish are often kept in laboratories conflict with their natural preferences [21]. The present study pertains to the information on microhabitat availability/ preference by zebrafish, *Danio rerio* [1] from Karala River system.

Materials and Methods

Study area

The fieldwork was conducted at Karala River **(26.52°N 88.73°E)**, popularly known as 'The Thames of Jalpaiguri which originates from the Baikunthapur forest and flows down into the Teesta near Mandal Ghat in Jalpaiguri Town, bisecting Jalpaiguri District located on the northern part of West Bengal state of India. Survey was conducted during the summer season, from December 2013 to May 2014, under clear and sunny. The total catchment area was 141 km^2, most of which is covered by arable land. The basin of this river sustains life and livelihoods of tea gardeners, fishermen and slum-dwellers. The present site (Karala River) was chosen based on its sufficient fish density so as to obtain enormous data on *Danio rerio* and to minimize the probability of making many observations of the same individuals. The river Karala presents heterogeneous physical properties and a wide range of available microhabitats with different characteristics, allowing for the differentiation between stochastic and selective use of the microhabitats.

Fish collection and habitat survey

Seines, rectangular hand nets and drag nets were used based on the habitat conditions. Local fisherman was also engaged for fishing in large bodies. Quantification of habitat characteristics and habitat inventory were carried out by adopting standard methods [46,47]. Inventory was carried out at a fixed point, which was designed as a reference point. In each stream point, 100-250 m reach was quantified for habitat, substratum, depth, flow, fish cover and distance from bank characteristics. A number of 10-25 transects, were taken across the stream channel, depth, water velocity and dominant substrates were measured at 0.5^{-1} m intervals across transects. Twenty transects that were perpendicular to the water flow were established 10 m apart from each other along 200 m of the study site. At each transect, five microhabitat parameters were recorded in 2.5 × 2.5 m (6.25 m^2) quadrats. The resources available in the study site were quantified in 40 quadrats, with a total of 250 m^2, established within the total sampling area of 400 m^2. The water velocity was measured using electronic flow meter (propeller type). Water velocity was grouped into five categories (F1-F5): stagnant, very slow, slow, moderate and fast corresponding to 0; 0-0.15; 0.16-0.30; 0.31-0.60 and >0.60 m.sec-1 respectively. The depth measurement was used to determine the proportion of the habitat within five depth categories (D1-D5) corresponding to 0-10, 11-25, 26-40, 41-60 and >61-100 cm, respectively. Substrate categories were identified visually according to particle size as bedrock (>512 mm diameter), boulder (128-512 mm), cobble (64-128 mm), gravel (16-64 mm), sand (1-16 mm) and leaf litters. Fish cover was classified into six categories: No cover, root undercut, sand undercut, boulder undercut, submerged log and overhanging vegetation. The riparian cover in the site was estimated using spherical densiometer (Model: C).

Statistical analysis

The obtained data were statistically analyzed using GraphPad Prism version 5.0 for Windows, San Diego California, USA. The data of this work were presented as mean values. In order to make comparison between the available and most preferred environmental variables of the zebrafish, one-way ANOVA was performed. The variable covariates in each analysis $P<0.05$ was taken as significant. Canonical Correspondence Analysis (CCA) was performed to determine most preferred environmental variables and respective components of the zebrafish. The Principal Component Analysis (PCA) was used to identify the preferred variations among the habitat characteristics. The CCA and PCA were performed using the freely available statistical packages PAST ver.2.17 [48].

Results and Discussion

Microhabitat availability, utilization and habitat preference

The Karala river was characterized by stagnant to fast flowing water (Figure 1). Sand, gravel and cobble were the dominant substrate types. Most of the sampled sites were shallow (>60 cm). Riparian vegetation was present at most sites. Water temperature was <18°C in most sites during the sampling periods. Microhabitat availability was quantified along the study site. The analysis of microhabitat availability revealed that the studied site was quite heterogeneous (Table 1). A total of 756 adult individuals were observed the length varying from 22.3-37.8 mm (average of 25.7±0.4 mm). D. rerio was observed to be inhabiting the water column, organized in shoals (60% of the observations) and on average, 15cm from the riverbank. The individuals were frequently in groups (up to 15-30 individuals) swimming in the water column and spending much of their time foraging in environments with moderate currents. Only adult individuals (>22.0) were observed in the study site.

D. rerio was found to be significantly associated with substrate, depth and velocity $P<0.05$. High selection probabilities were for coarse substrate types (pebble and boulders) and shallow depth (<50 cm). Moderate flow and fast flow were the most preferred velocities when compared to slow flow.

Data on availability and use of microhabitats such as substratum, flow, depth, habitat, fish cover and distance from bank were used for analyzing the habitat availability/preference of *D. rerio* in the studied stream (Figure 2). In the microhabitat of substratum, *D. rerio* mostly preferred sandy areas, the mean availability was 44.4% and the mean utilization was 55.5% which had significantly higher availability/usage (f=44.5; df=2.431; p=0.0555) when compared to gravel, cobble, boulder, bedrock and leaf litter.

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Environmental Descriptors	Description	Use Measurement	Availability Measurement
Distance from the nearest bank (cm)	Distance from the stream bank to a given point in the stream channel, measured through a measuring tape	Distance from to the stream bank to the fish focal position	Distance from the stream bank to a place where the quadrats were placed
Water Column Depth (cm)	Distance from the water surface to the stream bottom	Distance from the water surface to the fish focal position	Distance from the water surface to a place where the quadrats were placed
Mesohabitat	Occurrence of the five mesohabitats (pools, plunge pool, isolated pool, runs and riffles) present in the study site	Kind of mesohabitats predominating in the fish focal position	Mesohabitat types predominating in a place where the quadrats where placed
Water Velocity (V=m/sec)	Water velocity measured through an electronic flow meter (propeller type)	Water velocity measured at the fish focal position	Water velocity measured where the quadrats were placed
Substratum	Substratum types occurring in the study site: (i) Bedrock (particles >512 mm diameter), (ii) boulder (particles >128-512 mm), (iii) cobble (particles > 64-128 mm), (iv) gravel (particles 16-64 mm), (v) sand (particles1-16 mm) and (vi) leaf litters	Percentual of each substratum type measured just below the fish focal position	Percentual of each D16 substratum type measured just below the quadrats
Vegetal Cover	Quantity of instream vegetation serving as underwater refuge	Percentual of instream vegetation measured just above at the fish focal position	Percentual of instream vegetation measured just above the quadrats

Table 1: Microhabitat descriptors and their respective measurements for fish use and environmental availability measurements applied for the microhabitat study of *Danio rerio* from Karala river.

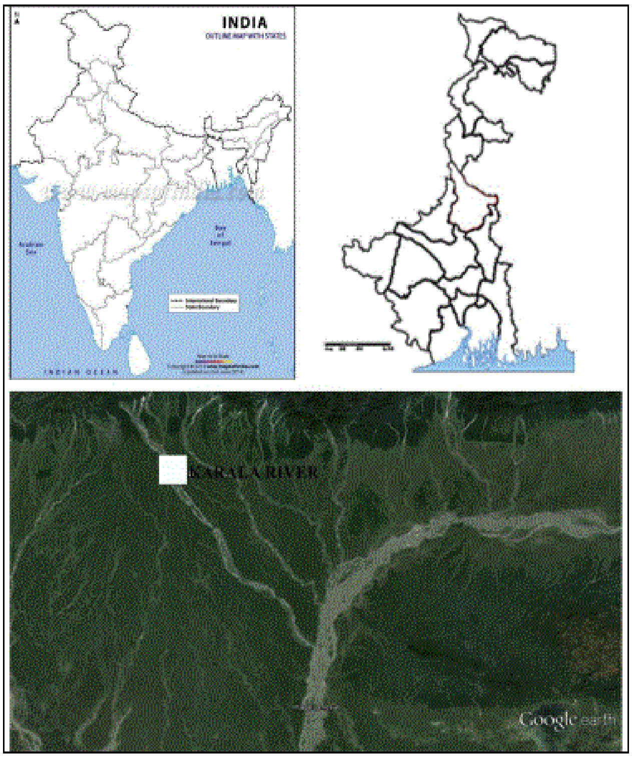

Figure 1: Map showing the study sites of Karala river of Jalpaiguri district, Northern Bengal, India.

During the flow characteristics studies, *D. rerio* mostly preferred very slow flow and slow flow areas with the mean availability of 14.4 and 28.9%, respectively and the mean utilization was 29.8 and 28.0%, respectively which had significantly higher availability/ usage (f=6.665; df=2.057; p=0.0129) compared to stagnant, moderate and fast flows. During the depth character studies, D. rerio mostly preferred 1 and 2 depth areas and the mean availabilities were 22.2 and 26.6%, respectively and the mean utilization was 28.6 and 31.5%, respectively which had significantly higher usage mean (f=7.61; df=2.363; p=0.0001) when compared to the depths 3, 4 and 5. During the habitat characteristics analysis, *D. rerio* mostly preferred run, pools, isolated pool and riffle areas with mean availability of 36, 18.2, 19.7 and 19%, respectively and the mean utilization were 25.6, 24.4, 23.1 and 21.3%, respectively which had significantly higher usage mean (f=7.61; df =2.363; p=0.0001) when compared to plunge pool.

Canonical correspondence analysis

In order to determine the preferred association of *D. rerio* to known microhabitat variables the Canonical Correspondence Analysis (CCA) was performed. The CCA showed significant association between *D. rerio* with relative preference and the microhabitat variables such as the habitat, flow, substratum, depth, fish cover and distance from the nearest bank (Monte Carlo permutations, P<0.05. Of the thirty two microhabitat variables analysed, the forward selection procedure positively correlated with eighteen environmental variables for the CCA ordination. The first two axes were used in the interpretation of results for the respective ordinations. The ordination plots of species scores indicated their relationship with reduced number of environmental variables (Figure 3). *D. rerio* mostly associated by the ordination represented variables with habitat parameters such as Pool (P), Plunge pool (PP) and Isolated pool (IP); flow parameters such as Slow flow (SL), Moderate flow (MOD) and Fast flow (F); the substratum parameters such as Sand (S), Gravel (GRA) and Leaf litter

(LL); depth such as Depth 1 (D1), Depth 2 (D2), Depth 3 (D3) and Depth 5 (D5); fish cover parameter such as root undercut (RU) and distance from bank parameters such as Distance from bank 1 (DB1), Distance from bank 2 (DB2), and Distance from bank 3 (DB3). CCA revealed a clear separation of the *D. rerio* along microhabitat variables.

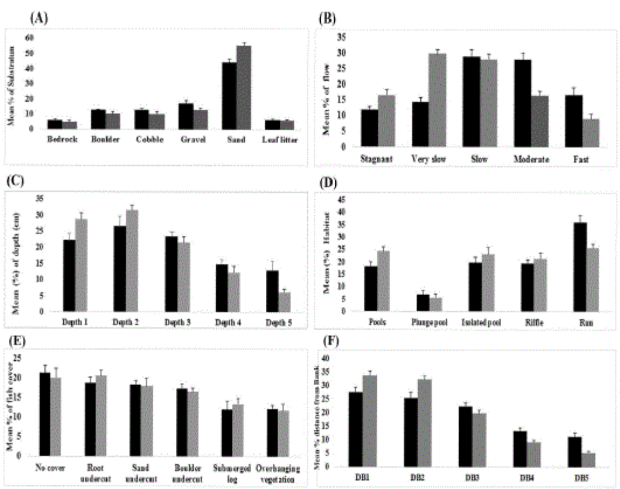

Figure 2: Availability (black bars), use (grey bars) for the five studied microhabitat parameters affecting *Danio rerio* from Karala river : (A) Mean % of substratum; (B) Mean % of flow; (C) Mean % of depth; (D) Mean % of habitat; (E) Mean of fish cover; (F) Mean % of distance from the nearest bank.

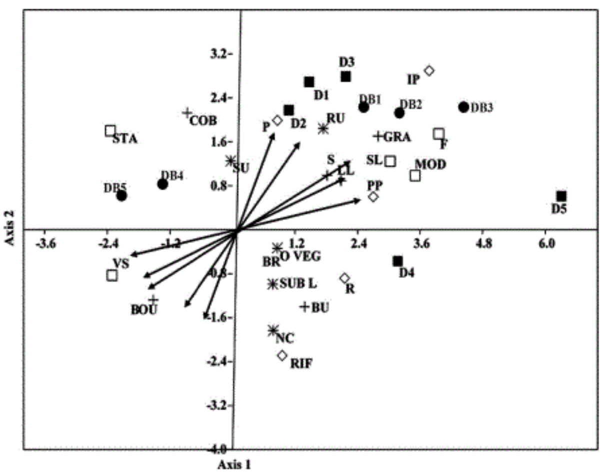

Figure 3: Canonical correspondence analysis (CCA) based on the habitat, flow, substratum, depth, fish cover and distance from the nearest bank. Bedrock (BR); Boulder (BOU), Cobble (COB); Gravel (GRA); Sand (S); Leaf litter (LL); Stagnant STA); Very slow (VS); Slow (SL); Moderate (MOD); Fast (F); Depth 1-5 (D1, D2, D3, D4, D5); Pools (P); Plunge pool (PP); Isolated pool (IP); Riffle (RIF); Run (R); No cover (NC); Root undercut (RU); Sand undercut (SU), Boulder undercut (BU); Submerged log (SUB L); Overhanging vegetation (O VEG); Distance from Bank 1-5 (DB1, DB2, DB3, DB4, DB5).

Zebrafish have been found to distribute all over India covering a variety of habitats [32]. Common typical characteristics of their habitats are low water flow, ground covered with sand, silt or pebbles and overhanging vegetation. Such habitats are frequently found in secondary or tertiary channels connected with the main channel of a river or adjacent to wetlands and paddy fields [31]. Zebrafish appears to be a floodplain species rather than a true riverine species as they are most commonly encountered in shallow ponds and standing water bodies, often connected to rice cultivation [40]. Spence [43] found no zebrafish in either rivers or temporary creeks that opened during the monsoon season, where zebrafish are found in streams and rivers, these typically have a low flow regime and zebrafish have been most often encountered at the margins [22,31]. Behavioral observations of their vertical distribution indicated that they occupy the whole water column and occurs frequently in open water as amongst aquatic vegetation [43].

Principal component analysis

In order to determine the target species *D. rerio* for its preferred association to particularly microhabitat parameters, the Principal Component Analysis (PCA) was performed. The PCA revealed a clear separation of the fish species richness along water quality and habitat characteristics (Figure 4). A total of 10 components were extracted and the first three components with higher eigenvalue accounted for about 98.46% of the total variances. The first component axis alone explained 86.33% of the variance with an Eigenvalue of 5.3 with high loadings (> 0.7). The second and third components explained 8.91 and 3.22% of variance with an Eigenvalue of 0.8 and 0.6, respectively. Among the 32 microhabitat parameters, the PCA ordination plot could be seen that sets of microhabitat variables separated clearly. Two groups on the right side of the ordination, the upper one are more associated with depth parameters D2, D1, and D4; flow parameters such as slow flow, moderate flow and fast flow; and the distance from bank parameters such as DB1, DB2 and DB3. The lower one consisted of the fish cover parameter such as Root undercut; the habitat parameters such as isolated pool, pool and plunged pool. On the left side of the ordination the upper one are the substratum characteristics such as sand, leaf litter and gravel.

Partitioning of habitat or other resources is the mechanism for the co-existence of different species in the same environment [49] and the tropical fish assemblages are more structured and are well segregated in their food usage with the great diversity of fishes in the streams of India, the diversity is likely to be correlated with or associated with habitat segregation and within this it is based on feeding microhabitats related to their behaviors and anatomical adaptations [50].

Zebrafish share their habitat with a variety of other fish as that may act as competitors for food, including the Cyprinids like *Puntius*, *barbs*, other *Danio* species and especially *Esomus danricus*, which has a similar size and gape and occupies similar positions in the water column [31]. Their gut content analyses suggests that zebrafish mainly feed on allochthonous materials (i.e., not deriving from their habitat), such as red ants or other terrestrial insects that have fallen into the water, but also on aquatic insect larvae and crustaceans as well as phyto and zooplankters [34].

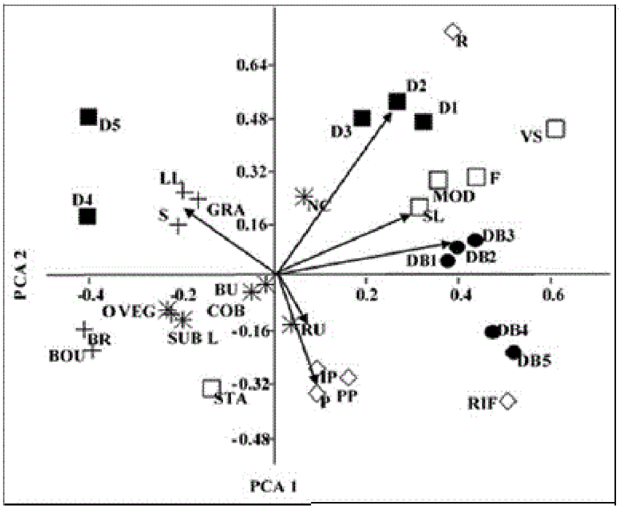

Figure 4: Principal component analysis (PCA) based on the habitat, flow, substratum, depth, fish cover and distance from the nearest bank. Bedrock (BR); Boulder (BOU), Cobble (COB); Gravel (GRA); Sand (S); Leaf litter (LL); Stagnant (STA); Very slow (VS); Slow (SL); Moderate (MOD); Fast (F); Depth 1-5 (D1, D2, D3, D4, D5); Pools (P); Plunge pool (PP); Isolated pool (IP); Riffle (RIF); Run (R); No cover (NC); Root undercut (RU); Sand undercut (SU), Boulder undercut (BU); Submerged log (SUB L); Overhanging vegetation (O VEG); Distance from Bank 1-5 (DB1, DB2, DB3, DB4, DB5).

Conclusion

The *D. rerio* was found to be generally eurythermal, and the abundance of riparian cover did not significantly influence habitat association. Substrate and depth have significant variables. The *D. rerio* showed a greater probability of use for a substrate size range of 300-400 mm and a depth range of 0-40 cm. The preference of *D. rerio* in the studied streams viz: pool, plunge pool and isolated pool; slow flow, moderate flow and fast flow; and the substratum parameters: sand, gravel and leaf litter; depth parameters like D1, D2, D3 and D5; fish cover parameters such as root undercut and distance from bank parameters such as DB1, DB2 and DB3. Differences in the use and availability/usage of various microhabitat descriptors revealed non-stochastic patterns of microhabitat use by *D. rerio*.

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