RUHUNA JOURNAL OF SCIENCE
Vol. 4, September 2009, pp 51-64
http://www.ruh.ac.lk/rjs/rjs.html
ISSN 1800-279X
DOI: http://doi.org/10.4038/rjs.v4i0.59

Morphological variation of Puntius bimaculatus (Cyprinidae) with respect to altitudinal differences and five major river basins of Sri Lanka

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Abstract. Forty sites located in five major river basins (Mahaweli, Kelani, Kalu, Gin, and Nilwala) at four different altitude ranges (0-150 m, 151-300 m, 301-600 m and 601-1200 m) were sampled to study the distribution and intra-specific morphological variation among the endemic fish species *Puntius bimaculatus* populations in Sri Lanka. Twenty one (21) morphometric characters and 15 meristic characters were recorded with respect to each specimen. Size adjusted meristic data and non transformed meristic data were analysed. In addition, fourteen physico-chemical parameters were recorded from each site.

Puntius bimaculatus was found only at 10 locations. Out of the total of 63 individuals 57% were collected from the highest elevation. Twelve meristic and 14 morphometric characters of fish were significantly different among the five rivers. Ten meristic and morphometric characters were significantly differed among altitudinal ranges. Discriminant function analysis resulted three discriminant functions for morphometric characters explained 74.8%, 16.4%, 8.8% variance of the data set. They classified the originally grouped cases (according to the altitudinal range) with an accuracy of 87%. Length of the caudal fin, maximum depth of the fish and the position and length of the dorsal fin highly contributed in discrimination of fish into their respective altitude ranges. Meristic characters also resulted in three discriminant functions and explained the variance of 62.3%, 30.9% and 6.8%. They classified originally grouped cases with a 100% accuracy showing that meristic characters are powerful in discriminating the fish. Rays in the ventral, pelvic, caudal and anal fins, and number of scales on lateral line, number of pre and post dorsal scales were the major meristic characters contributed to the discrimination of fish. Most of the physico-chemical parameters were also significantly different with respect to the altitude ranges and rivers. Clear intra-specific morphological variations observed among individuals from different altitudinal ranges and rivers could be an adaptation to their habitats with diverse environmental conditions.

Introduction

Morphological plasticity according to environmental variability is commonly found among many fish species, predominantly in freshwater fish species. Phenotypic variation according to environmental variability has been widely used by ichthyologists to differentiate among species and among populations within a species (Ihassen *et al*. 1981; Tudela 1999; Murta 2000). Effect of environmental factors on fish morphology also well documented (Hinder and Jonsson 2003; Peres-Neto and Magnan 2004; Grunbaum *et al*. 2007).

Morphological variability of fish is considered to be an important adaptive strategy for populations experiencing inconsistent environments (Stearns 1989, Wimberger 1991, Scheimer 1993) Variability of environment could be explained by abiotic components such as physico chemical parameters of water, habitat and substrate types and biotic components like competition and predation, which serve as selective pressures. Most of the abiotic components in an environment are determined by geographic location such as altitudinal and latitudinal position where the species inhabit. Therefore, altitudinal variation could indirectly affect the morphology of a species. Elavational variation has been shown to be correlated with body size (Endler 1977; Atkinson and Sibly 1997; Jin *et al.* 2007), and skull size (Liao *et al.* 2006). However, such studies are mainly focused on endothermic vertebrates and only a few studies have addressed the relationship of altitudinal variation on fish morphology (Baum *et al.* 2004; De Silva *et al* . 2006)

Endemic freshwater fish species *Puntius bimaculatus* (Bleeker 1863) is commonly known as the redside barb. Small size, beautiful colouration and hardy nature have attributed this species as an important species in aquarium trade. *P. bimaculatus* is reported in five major river basins (Mahaweli, Gin, Nilwala, Kelani and Kalu) in Sri Lanka and also show a wide distribution. According to Schut *et al.* (1984), this species was reported to be found in montane rocky streams above the flood levels of 150-300 m altitude range. Pethiyagoda (1991) recorded that *P. bimaculatus* was found even in upper elevations as 1500 m where other *Puntius* species had not been recorded. However, human activities mainly habitat degradation, exploitation for aquarium trade and pollution have narrowed the distribution boundaries of the species and their abundance. Pethiyagoda (1991) also noted that *P. bimaculatus* showed intra-specific differences in morphology between populations, but detailed information is scarce.

As a result of anthropogenic activities the status of *P. bimaculatus* has become threatened. In order to reduce the risk of extinction, conservation strategies are needed to be formulated. As such, distribution patterns of the species and identification of morphologically distinct groups are important. Therefore, present study was focused on to identify the distribution of *P. bimaculatus* with respect to altitudinal ranges and rivers, to investigate the variability in morphology according to altitudinal ranges and rivers, and to identify a set of characters that mainly contribute to these variations.

Matrials and methods

This study was conducted as a part of a large scale sampling programme which covered forty sites in five major river basins (Mahaweli, Gin, Nilwala, Kelani and Kalu) of Sri Lanka representing four different altitude ranges (0.150 m, 151-300 m, 301-600 m and

601-1200 m). Study sites were selected along the river starting from closer to the head waters to downstream lowlands. Table 1 shows the number of sampling locations in each river. Fishes were caught by gape nets, cast nets and scoop nets.

Thirty six morphological characters (morphometric and meristic) were scored with respect to each individual. Morphometric measurements (21) consisted of total length (TL), standard length (SL), fork length (FL), maximum body depth (MBW), head length (HL), eye diameter (ED), post orbital length (POL), dorsal fin length (DFL), pre dorsal length (PDL), post dorsal length (PODL), anal fin length (AFL), pre anal length (PAL), post anal length (POAL), pre ventral length (PVL), post ventral length (POVL), pre pelvic length (PPL), post pelvic length (POPL), caudal fin length (CFL), width of the caudal fin when fully spread (CSPR), caudal peduncle height (HCPD) and length from the end of the dorsal fin to end of the caudal peduncle length (LCPD).

Fifteen meristic characters scored were: number of lateral line scales (lls), number of transverse scales (tr), number of pre dorsal scales counted from the edge of the operculum to the beginning of the dorsal fin (prds), number of post dorsal scales counted from the end of the dorsal fin to the beginning of the caudal fin (psds), number of dorsal fin scales counted from the beginning of the dorsal fin to the end of the dorsal fin (dfsc), scales around the caudal peduncle (cped), number of dorsal fin rays (dfr),number of anal fin rays (afr),number of pelvic fin rays (pfr),number of caudal fin rays (cfr) number of ventral fin rays (vfr) number of dorsal fin spines (dfs), number of anal fin spines (afs), number of pelvic fin spines (pfs) and number of ventral fin spines (vfs).

Linear measurements were made using vernier calipers to nearest 0.01 mm. Stereo microscope (Wild M5A) and hand lenses were used to determine meristic counts. Physico- chemical parameters *viz*. temperature, pH, salinity, alkalinity, conductivity, total suspended solids, inorganic suspended solids, organic suspended solids, dissolved oxygen, biological oxygen demand, chemical oxygen demand, nitrates (NO_3), phosphates (PO_4) and chlorophyll-a of water at each location were obtained using standard methodologies. These parameters were taken only once at the time of collection of the fish sample.

Statistical analyses were carried out separately for morphometric and meristic characters. All the morphometric variables were corrected for size by converting them as ratios of standard length (Austin and Knot 1996). Some selected characters also presented as a ratio of another related morphometric character (Table 2). Meristic characters were used as raw data as they have been reported as independent of fish size (Barlaw 1961, Tudela 1999, Murta 2000).

To study the relationships between morphology and altitudinal range and river both univariate and multivariate statistical methods were followed. Data of individuals of different rivers but occupied the same altitude range were pooled to investigate altitudinal variation of morphology. As the size adjusted morphometric data and meristic data were not normally distributed even after logarithmic transformation, Kruskal-Wallis non parametric test was performed. A multivariate technique, Discriminant Function Analysis (DFA), was performed to investigate the integrity of pre defined groups (according to altitudes) and to determine a set of characters which are mainly responsible in separating the individuals to different altitude ranges. This analysis is robust to deviations from normality (Sokal and Rohlf 1995, Claytor *et al.* 1991). Kruskal-Wallis test was performed to identify the relationship between physico chemical parameters and the altitudinal level. Pearsons correlation was used to identify the parameters which are highly correlated with the altitude range and the river. All calculations were carried out with the statistical software, SPSS version 16.

Results

From the 40 locations sampled, *P. bimaculatus* were found only in 10 (25.5%) sites from where a total of 63 fish were collected. At some locations fish were scarce and only one or two fish were found. Only a representative sample of the fish was collected from each location where the fish were present in abundance. Table 1 shows the river, sampling sites (with the altitude range) and the number of individuals collected. Fifty seven percent (57%) of fish caught were from the highest elevation range 601-1200 m. *P. bimaculatus* were not found in all four elevations in a single river basin. Fish were recorded in all elevations of Mahaweli river except the lowest. Only one site each of the Kelani and Gin rivers and two sites each of the Kalu and Nilwala recorded the *P. bimaculatus.* Sampling site at Ingiriya situated in the lowest elevational range is the only site which recorded a single fish.

Table 1 River, sampling sites (with the altitude range) and the number of individuals collected

River	Total number of sites sampled	Number of sites *P. bimaculatus* recorded (%)	Sampling site and altitude (m)	No of fish collected
Mahaweli	10	40%	Pallegama (301-600) Arawa (151-300) Welimada (601-1200) Demodara (601-1200)	02 06 12 13
Kalu	09	22%	Ingiriya (0-150) Bopath Ella (0-150)	01 08
Kelany	12	8%	Rampadeniya(601-1200)	02
Gin	05	20%	Viharahena (601-1200)	09

Nilwala	04	50%	Deiyandara (151-300) Mawarala (301-600)	02 08
Total	40	25%		63

From 22 morphometric character ratios (Table 2a), 14 (63.6%) were significantly different among five rivers and 10 characters (45.5%) were significantly different among four altitude ranges. Among these 09 characters were common to both river and the altitude ranges. From the 15 meristic characters 12 (75%) and 10 (66%) characters, were significantly different among rivers and altitudinal ranges respectively and 09 were common to both river and altitude ranges (Table 2b). Comparing the number of significantly different characters obtained, meristic characters showed higher number compared to morphometric characters.

Table 2a. Morphometric Characters showing significant differences among rivers and altitude ranges.

Morphometric Character (as a ratio)	*Asymptotic significance by river*	*Asymptotic significance by altitude range*
*FL/SL***	*0.010**	*0.006**
*MBW/SL***	*0.000**	*0.040**
*ED/HL***	*0.000**	*0.031**
HL/SL	*0.126*	*0.321*
POL/SL	*0.119*	*0.002**
DFL/SL	*0.261*	*0.770*
PDL/PODL	*0.005**	*0.092*
PDL/SL	*0.217*	*0.140*
PODL/SL	*0.003**	*0.192*
AFL/SL	*0.003**	*0.112*
PAL/SL	*0.131*	*0.408*

POAL/SL	*0.001**	*0.332*
PAL/POAL	*0.003**	*0.315*
PVL/POVL	*0.667*	*0.945*
PVL/SL	*0.354*	*0.072*
POVL/SL	*0.063*	*0.484*
*PPL/POPL ***	*0.002**	*0.001**
*PPL/SL***	*0.000**	*0.001**
*POPL/SL***	*0.015**	*0.006**
*CFL/SL***	*0.029**	*0.001**
*CFL/CSPR ***	*0.000**	*0.000**
*HCPD/LCPD***	*0.002**	*0.000**

- significant at 0.05 level, ** significantly different characters common to both river and the altitude range,

Table 2b Meristic Characters showing significant differences among rivers and altitude

Meristic character	*Asymptotic significance by river*	*Asymptotic significance by altitude range*
*lls***	*0.000**	*0.000**
*tr***	*0.001**	*0.000**
cped	*0.022*	*0.013**

dfs	*0.001**	*0.180*
dfr	*0.000**	*0.667*
afs	*0.000**	*0.360*
*afr***	*0.012**	*0.000**
vfs	*nv*	*nv*
*vfr***	*0.000**	*0.000**
pfs	*nv*	*nv*
*pfr***	*0.000**	*0.002**
*cfr***	*0.000**	*0.000**
*prds***	*0.000**	*0.000**
*dfsc***	*0.019**	*0.026**
*psds***	*0.005**	*0.000**

- significant at 0.05 level, ** significantly different characters common to both river and the altitude range; nv –no variation

Three canonical discriminant functions having Eigen values 1.985, 0.436 and 0.232 were obtained by DFA of morphometric characters according to the elevation. These three functions were able to explain the 74.8%, 16.4% and 8.8% of total variance respectively.

Table 3a Major morphometric characters contributed for the discrimination and coefficients obtained for each canonical discriminant function.

Character	*Function*		
	1	*2*	*3*
MBW/SL	*-.750*	*.287*	*.488*
POL/SL	*-.493*	*-.553*	*.052*

PDL/PODL	-1.117	.411	-.821
PDL/SL	1.373	-.392	-.127
PVL/SL	.127	.675	-.017
PPL/SL	.574	-.485	.500
CFL/CSPR	.805	.092	-.048

Table 3b Major meristic characters contributed for the discrimination and coefficients obtained for each canonical discriminant function

Character	Function		
	1	2	3
lls	-.197	.466	.717
afs	-.088	-.560	.625
afr	-.333	-.622	.227
vfr	1.127	-.437	-.062
pfr	1.080	.365	-.006
cfr	.579	1.145	.008
prds	-.264	-.585	.305
dfsc	.032	.293	.580
psds	.526	.590	.395

From the seven characters contributed mostly for the discrimination (Table 3a), length and the position of the dorsal fin (determined by PDL/PODL and PDL/SL), maximum depth of the body (MBW) and relative length of the caudal fin (CFL/CSPR) mainly correlated with first discriminant function. POL/SL (negative correlation) and PVL/SL (positive correlation) associated with the second function.

Results of the DA of meristic characters show that 09 characters (Table 3b) highly contributed to separate the fish into four altitudinal ranges. These characters were able to extract three canonical discriminant functions having Eigen values 10.631, 5.275 and 1.165 explaining 62.3%, 30.9% and 6.8% the variance respectively (Table 3b). Among these characters, number of rays found in the ventral, pelvic, caudal and anal fins shows the highest (positive) correlations. Position of the dorsal fin which could indirectly explain by prds and psds mainly contributed to the second discriminant function. Lateral line scales showed the highest positive correlation with the third function.

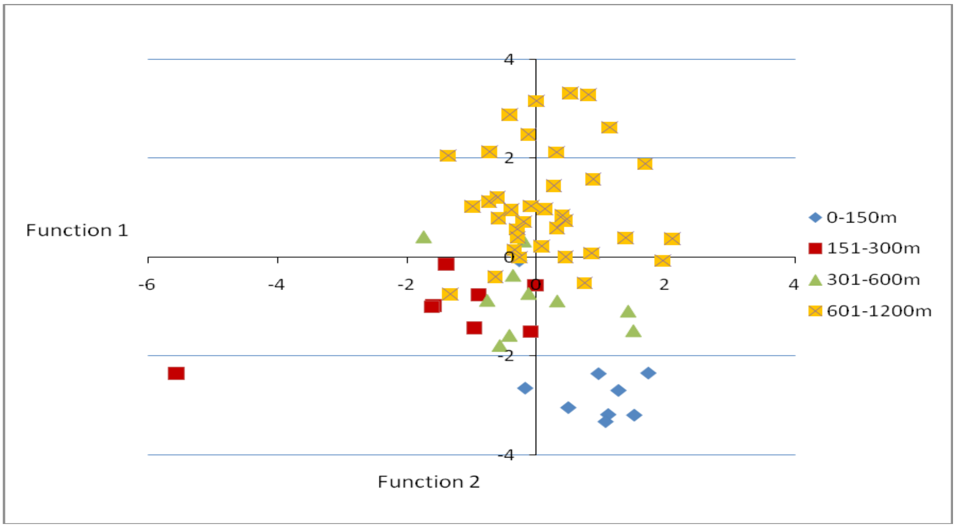

Figure 1. Plot of discriminat scores obtained for Discriminant Function 1(DF1) and Discriminant Function 2 (DF2) for each individual for morphometric characters

Plotting the discriminant scores of fish obtained for DF1 and DF2 showed clear separation according to the altitude range (Figures 1 and 2). However, this separation is very different from meristic characters. The overall percentage of fish correctly classified for according to the discriminant functions of morphometric and meristic characters given in the analysis were 85.7% and 100%, respectively.

All physico chemical parameters (except pH and PO_4 in different altitude ranges) were significantly different among the rivers as well as altitude ranges. All parameters showed significant correlations (negative or positive) (Table 4) among rivers. Most of the chemical factors were significantly correlated with the altitudinal range where less number of physical factors showed significant correlation. However, it should be noted that these parameters were taken only once at the time of collection of samples and therefore time periods of taking physico chemical samples among all locations were not identical.

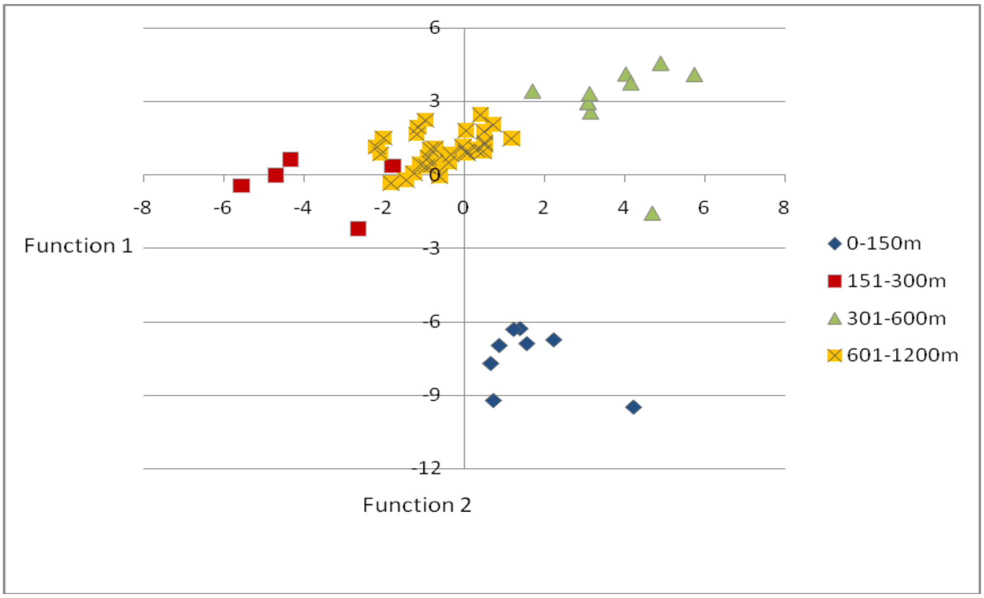

Figure 2 Plot of discriminat scores obtained for DF1 and DF 2 for each individual for meristic characters

Table 4 Physico chemical parameters and their correlations among altitude ranges and rivers

Parameter	*correlation (among altitudes)*	*Correlation (among rivers)*
Temperature	*-0.766*	*0.31***
pH	*-0.065*	*0.850*
Salinity	*0.476***	*0.280**
Alkalinity	*-0.291**	*0.734***
Conductivity	*0.195*	*0.788***
Total Suspended Solids	*0.215*	*0.697***

Inorganic Suspended Solids	*0.265**	*0.638***
Organic Suspended Solids	*0.026**	*0.829***
Dissolved Oxygen	*-0.481***	*-0.510***
Biological Oxygen Demand	*-0.428***	*0.689***
Chemical Oxygen Demand	*0.406***	*0.943***
NO3	*0.139*	*0.952***
PO4	*0.355***	*0.368***
Chlorophyll content	*0.052*	*0.937***

**. Correlation is significant at the 0.01 level (one-tailed).*. Correlation is significant at the 0.05 level (one-tailed).

Discussion

Present study demonstrated an intra specific morphological variation of the individuals among five rivers and in different altitude ranges. This variation is markedly shown in meristic characters compared to morphometric characters. Studies have shown that morphometric characters are more suitable than meristic characters to describe intra specific variation (Murta 2000, Costa *et al*. 2003). However, in some studies meristic characters have been successfully used for stock identification (Misra and Bowering 1984, Melvin *et al*. 1992). Better separation (in 100% accuracy) of fish according to four altitudinal ranges by meristic characters show that they are more suitable in discrimination of altitudinally different fish groups.

Higher percentage of *P. bimaculatus* was found in the highest elevation and most of these locations were rocky hill streams. Kortumulder (1987) has also shown that juvenile *P. bimaculatus* were found in hill marshes above flood levels and adults were mainly found in the rocky hill streams having high gradient (2.5% or more) where they favoured streams with fast current. Hill marshes being the spawning habitats of the fish and nursery area of the juveniles and connectivity of the hill marshes to the hill streams may have favoured the abundance at higher elevations. Relatively unpolluted and undisturbed nature of the sampled locations situated at high elevations may also have contributed to the high abundance.

Most physico -chemical parameters have shown significant differences and high correlations (negatively or positively) with altitudinal range. Morphological plasticity observed in *Puntius bimaculatus* might be due to an adaptive response to inhabit these changing environments.

Meristic characters are fixed in early embryonic life of the individual and remain unchanged thereafter. Thus, they respond to environmental factors only a short time period during embryonic development. These could result in wide variations among members of the same and even in different year classes of a single stock (Lindsey 1988). In contrast, morphometric characters varied according to the changing environmental conditions of the habitat throughout their life and the phenotypic plasticity have been shown in many freshwater fish species (Haindar and Jonsson 1993, Peres-Neto and Magnan 2004).

The meristic characters mainly contributed to discriminate the fish into four altitude ranges are number of scales (lls, prds, dfsc, psds) and number of fin rays (afr,vfr,pfr,cfr). Number of lateral line scales, dfsc and psds increase with the increase of altitude range. It is an indication of having longer bodies in higher altitudes.

Negative discriminant coefficients obtained for MBW.SL and positive coefficients for CFL.CSPR, indicate that the fish inhabiting in higher elevations tend to have elongated bodies. High water currents prevailing in these habitats (mainly rocky hill streams) possibly resulted in these changes to resist the high water velocity. PDL.PODL and PDL.SL and PVL.SL determine the position of the dorsal fin and ventral fins. Fins provide stability and maneuverability to the fish. Fish in high altitudes had more posteriorly placed dorsal fins . Dorsal fin serve to protect the fish against rolling, assists in sudden turns and stopping (Lindsay 1975). Hydrodynamics of the habitats that fish occupy may have affected the differences observed in the position of the fins.

With regard to the four altitudinal ranges and five different rivers significant differences in morphology were observed. Altitudinal differences in morphology in the fish belonging to the same river were not investigated as fish were not recorded in some altitude ranges. Even considering the small sample sizes which represented different altitudes for this study, morphological variations detected were significant to differentiate the individuals.

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