Journal of Aquatic Biology & Fisheries, Vol. 2(1) 2014: 290-296

MORPHOMETRIC AND ELECTROPHORETIC STUDIES OF *RASBORA DANDIA* (VALENCIENNES) (PISCES CYPRINIDAE) FROM VARIED HABITATS IN KERALA

Suvarna Devi, S.* and Rita Kumari, S.D.
Dept. of Aquatic Biology & Fisheries, University of Kerala, Thiruvananthapuram.
**Corresponding author: suvarna@asianetindia.com*

Received on: 18.06.2013, accepted on: 12.11.2013

Abstract: Morphometric study of cyprinid fish *Rasbora dandia* (Valenciennes) from a lentic and two lotic habitats in Kerala revealed significant differences in morphometric parameters like head length, body depth, predorsal length, dorsal fin length, pectoral fin length and pelvic fin length, indicating morphological heterogeneity of the species. SDS- PAGE of eyelens protein was employed to examine the discreteness of populations within the species, which showed similar banding pattern from all three habitats. Despite the different level of significance between various body features, homogeneity is not disturbed as there is no genetic variation of *R. daniconius* from three geographically isolated locations. The significant variations in morphometric studies can be attributedonly to environmental differentiation.

Key words: Morphometry, electrophoresis, heterogeneity, homogeneity, eye lens protein

INTRODUCTION

Cyprinids of the genus *Rasbora* are tiny to small elegant fishes inhabiting a rich variety of habitats from lakes to lower reaches of mountain streams and are widely distributed in the oriental region. These hardy and peaceful fishes are observed in small schools in the upper layers of flowing and standing natural ecosystems in India.The rasboras are good forage fishes that can be used in culture operations in carnivorous fishes. They are considered as effective larvicidal fishes and are widely used as experimental laboratory animal. They are much prized aquarium fishes.

Of late, the genus *Rasbora* has become as object of attraction to taxonomists andthe Sri Lankan and south Indian species is now considered as *Rasbora dandia* (Silva *et al*., 2010). Population boundaries are largely determined by local environment, the strength of barriers to migration, and the organism's inherent dispersal abilities. The interaction among these and genetic factors determines the potential for evolutionary divergence, and is at the heart of our understanding of ecological adaptation and ongoing speciation processes. When individuals can easily cross barriers between suitable habitat patches, gene flow acts to homogenize most neutral genetic diversity andmay swamp the effects of any local phenotypic selection whereas low levels of migration may facilitate ecological divergence, even when selection is modest (Markert *et al.,* 2010).

Interhabitat differences in biological characters in *R.dandia* could be detected among members of the species from varied habitats (Suvarna, 1999).Biometric studies are helpful in arriving at a more precise criteria for taxonomic diagnosis and also for understanding evolutionary trends in the species (Klingeberg *et al*., 2003). Morphometry have been commonly used to identify the stocks of fish (Turan *et al*., 2004; Suneetha and Damayanthi, 2008).

Poly Acrylamide Gel Electrophoresis of eye lens nucleus of these fishes from varied habitats was employed to examine the discreteness of the population within the species. Martin and Pagare (2012) studied comparative electrophoretic

studies of lens proteins isolated from *P. ticto* and*R. daniconius* and concluded that though both the fishes belong to Cyprinidae family, the two fishes might have evolved independently.

It has been demonstrated in the Salmonidae by Crozier and Ferguson (1986) that the genetic differentiation is often paralleled by ecological, behavioural and morphological divergences. Based on this,the present study was undertaken with a view that whether the potential spatial isolation of the species has led to heterogeneity of the population of fishes from varied habitats and whether this has led to any genotypic differentiation.

MATERIALS AND METHODS

Samples of *R. dandia* were collected fortnightly for a period of one year from a lentic habitat, a pond located in Vellayani(HabitatI) and two lotic water bodies Chackai canal (Habitat II) and Kallar river (Habitat III) located inThiruvananthapuram district, Kerala. Habitat II is a fast flowing mountain stream, while habitat III is a slow flowing man-made canal receiving urban sewage. Sampling was performed using gill net and cast net.

A total of 523 fishes were collected from three habitats for morphometric analysis and was preserved in 5% formalin for further studies.The measurement of the different parts of the body was made to the nearest millimetre using a pair of fine divider was taken from the left side of the body along a straight line. Standard length, head length, depth of body, snout length, diameter of the eye, inter orbital distance, pre dorsal length, length of the dorsal fin, length of the pectoral fin, length of pelvic fin, length of caudal peduncle were the body measurements considered for the present study.Of these standard length, diameter of eye and inter orbital distance were related to head length and rest of the characters were related to standard length. Method of covariance analysis adopted by Snedecor and Cochran (1975)was employed to check whether the growth rates differ significantly between the fishes inhabiting three habitats.

For eye lens analysis samples were brought live from the three habitats to the laboratory ensuring minimum disturbance during transportation. The lenses were repeatedly washed in distilled water to prevent contamination of vitreous humor, retinal and choroid elements.An equal amount of 30mg eye lens wasfrom fishes of three habitats I, II and III were weighed in an electronic balance. Then the lenses were pooled into a clean petri dish containing 4^{0C} homogenising buffer. Homogenising buffer was prepared from the stock solution of Tris.Hcl (2x) and PMSF, Phenyl methyl sulfonyl fluoride (10x) made final working solution in a concentration of 150mM Tris.Hcl and 1µ M PMSF and the P^H was adjusted to 7.8. After pooling the eye lens were thoroughly minced with sterilised scalpel for the complete penetration of homogenising buffer. The tissue was homogenised in an electric homogeniser of teflon tip.

The samples were centrifuged at 13000xg at 40^0C for 30 minutes in C-24 Remi cooling centrifuge. To 20µl of supernatant, 50 µl loading 2x buffer was added and heated the sample at 100^0C for 3 minutes. The SDS (sodium dodecyl sulphate) digested samples were transferred into acid clean eppendrof vials.

Samples of equal total protein (60µg) were loaded on 8.5 polyacrylamide gel with 5% stacking gel using the buffer system described by Lammeli (1970). The molecular weight marker protein Albumin Bovine Serum 66000 Daltons (66kDa) was boiled in 2x loading buffer and loaded in 8.5% gel.Electrophoresis was carried out at a contrast current of 15mA in stacking gel and 30mA in separating gel. Electrophoresis was performed until the bromophenol blue dye front has run off the bottom of the gel. After electrophoresis gel was separated and stained in 0.25% Coomassie brilliant blue R-250 for two hours.

The stained gels were destained and stored in 10% glycerol (AR grade) until the documentation. The gels were illuminated and photographed in black and white film. The distance of each fraction from the origin was measured and its relative mobility (Rm) was calculated using the formula.

Rm = Total distance travelled by the protein band / Total distance travelled by the bromophenol blue

RESULTS

Analysis of Morphometry

Analysis of covariance for 11 characters of fishes (males and females) obtained from three selected habitats was computed and the results are presented in Table 1. For all the characters exceptthe length of caudal peduncle, the slopes are found to differ significantly between samples from different habitats. Hence it can be concluded that the growth rates of fishes in each habitat are significantly different.

Table 2 shows the results of ANCOVA of the head morphometrics in relation to head length from the three habitats. From the results it is clear that for snout length and diameter of eye, significant differences could not be observed in the slopes, indicating that the growth rates are similar for these characters between the samples from different localities. But differences in average size of these characteristics could be observed in fishes of the different habitats as elevation showed highly significant differences. In comparison of inter orbital distance with respect to head length there is no difference either in slopes or elevation, thus similar growth rates and growth pattern are discernible in fishes from the three habitats.

Photographs of electrophoretic gel of eyelens proteins of *R.dandia* from the three habitats were shown in Fig. 1. The intensity of staining and

Table 1. ANCOVA comparing each morphometric character with standard length among *R. dandia* from various habitats

Source		Among B	Sum of group Deviations	Diff among slopes(Fs)	Adjusted means	Error	Diff among adjusted means(Fe)
	DF	2	517		2	519	
Head Length	SS	6.9234	486.6329	Sig* 3.678	39.9422	493.5564	21.001
	MS	3.4617	0.9413		19.9711	0.9509	
Body Depth	SS	9.0489	568.6003	Sig* 4.114	78.3956	577.6494	35.218
	MS	4.5245	1.0998		39.1978	1.113	
Predorsal length	SS	41.2791	2017.275	Sig* 5.29	9.8286	2058.555	1.239
	MS	20.6395	3.9019		4.9143	3.9664	
Dorsal fin length	SS	8.3001	370.0479	Sig* 5.798	22.9151	378.3479	15.717
	MS	4.1501	0.7158		11.4571	0.7289	
Pectoral fin length	SS	5.6966	337.4419	Sig* 4.364	31.1009	343.1386	23.52
	MS	2.8483	0.6527		15.5505	0.6616	
Pelvic fin length	SS	7.1345	322.1755	Sig* 5.724	35.3936	329.3099	27.891
	MS	3.5673	0.6232		17.6968	0.6345	
Caudal peduncle length	SS	0.7861	476.8442	NS 0.426	6.5605	477.6303	Sig* 3.564
	MS	0.3931	0.9223		3.2803	0.9203	

Level of Significance * (5%) Fcal e" 2.99 (P<0.05)

Level of Significance **(1%) Fcal e"3.3 (P<0.01)

Table 2. ANCOVA comparing each morphometric character with head length of *R. dandia* fromvarious habitats

Source		Among B	Sum of group Deviations	Diff among slopes(Fs)	Adjusted means	Error	Diff among adjusted means(Fe)
	DF	2	517		2	519	
Snout length	SS	1.1358	161.8674	NS	8.0803	163.0031	**
				1.814			12.864
	MS	0.5679	0.3131		4.0402	0.3141	
Eye diameter	SS	1.4892	240.6637	NS	10.4494	243.1529	**
				1.6			11.198
	MS	0.7446	0.4655		5.2247	0.4666	
Inter orbital length	SS	0.8869	228.3451	NS	1.5178	229.2321	**
				1.004			1.718
	MS	0.4435	0.4417		0.7589	0.4417	

Level of Significance ** (1%)
Fcal e" 3.3 (P<0.01)

Table 3. Rm values of the protein bands of *R. dandia* from the three habitats

No: of bands	Total distance travelled by the protein band			Rm value
	Habitat I	Habitat II	Habitat III	
1	5.2	5.2	5.2	0.66
2	5.8	5.8	5.8	0.74
3	6.4	6.4	6.4	0.82
4	7.4	7.4	7.4	0.94

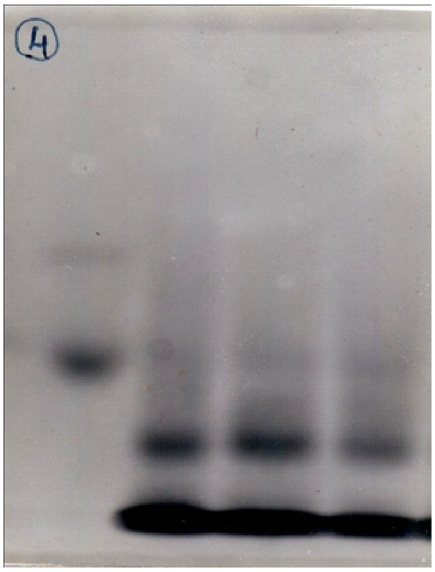

Fig. 1. Photograph of electrophoretic gel of eyelens proteins of *R. dandia* from the three habitats

relative mobility of different protein fraction can be seen in the figure.Similarities in relative mobility were considered as a criterion for the comparison of stocks from different localities. The differences in protein fraction were explained in terms of their mobility (Rm value) and their staining intensity.

The relative mobility(Rm values) of each fraction is given in the Table 3.Only four bands were obtained in the gel and respective bands were stained uniformly for each habitat.It is evident from the photograph that proteins of high molecular weight are totally absent. Since the marker protein has a molecular weight of 66,000 and gel was 8.5% concentration, the protein separated out in the present study has a molecular weight below that of the marker protein.

The present study reveals that the eye lens proteins of *R. dandia* from habitat I , II, and III showed identical protein profile.

Total distance travelled by bromophenol blue= 7.8

DISCUSSION

Variation in body shape can reflect ecological and behavioural differences. Because water is a densemedium, body shape particularly affects behavioural performance characteristics in fishes and other aquatic organisms (Webb, 1984).

The body shape of fishes can be expected to be of particular ecological and evolutionary

relevance. Accordingly, morphometric studies can provide useful information on the evolution of fishes (Schluter, 1993; Klingenberg and Ekau, 1996; Walker, 1997; Caldecutt and Adams, 1998; Douglas *et al*., 2001; Rüber and Adams, 2001; Hulsey and Wainwright, 2002).

Analysis of a set of phenotypic characteristics regarded as a more appropriate method than the use of a single character for determining morphological relationships between population of a species (Thorpe, 1987).This method has also been proposed as an efficient tool in management programme concerned with stock identification of freshwater fish species, and for investigating taxonomic problems in sympatric populations (Karakousis *et al*., 1991). The comparison of various body measurements between the habitats showed significant differences between the samples with regard to all body measurements. Except for the length of caudal peduncle, Fs were significantly different for all other characters. The growth rate of the three selected head characteristics in relation to head length was not significantly different for the samples between the three habitats, but a change in growth pattern was found to be significant.Morphometric characters can show high plasticity in response to differences in environmental conditions such as food, abundance, salinity and temperature. Morphological variation of the euryhaline cichlid fish *Etroplus suratensis* (Bloch) from six geographically apart estuarine localities along the southern and western coasts of Sri Lanka was studied by Suneetha(2007) and significant heterogeneity in morphology of the cichlid were found with respect to nine morphometric characters. The reasons behind such variability might include geographic isolation, phenotypic plasticity and local adaptation. Morphological characters are phenotypically plastic and are influenced each year by the physical environment during spawning and early juvenile stages (Austin *et al*., 1999).

Morphometric analysis of the entire Midas cichlid species complex across six crater lakes and both greatlakes revealed that Midas cichlids from each lake have their own characteristic body shapes (Elmer *et al*., 2010). Both *Amphilophus citrinellus* and *Amphilophus labiatus* have body shapes that are significantly different from their conspecifics in the neighbouring great lake and the differences among populations of *A. citrinellus* and *A. labiatus* between the two great lakes may indicate phenotypic divergence by drift promoted by long periods of geographical isolation.

A review of literature shows such intraspecific variation in both continuous and discontinuous variables have been reported in fishes. Gatz (1979) found intraspecific variation to exist between populations of sunfish within different streams in relation to body depth. Significant differences in all the six body measurements in samples of the clupeid fish *Esualosa thoracata* from east and west coast of India were recorded by Dutta and Rao (1981). Differences in discontinuous variables have also been reported earlier and these have been attributed to migratory behaviour, different ecological condition of the fish and to some accidental loss. Some of these variables were explained as temporary, due to different ecological condition and liable to disappear with the change of the habitat (Dube and Dubey, 1987; Piska, 1990).

Gatz (1979) during his study on community organisation in fishes based on morphological features investigated the possibility of intraspecific variation in morphology. According to him the observed differences probably involved adaptation to local habitat condition or to phenomena such as character displacement or the founder effect. In the present study, as all the characters showed intraspecific variations within different habitats, both the explanations of Gatz cannot be ruled out.

Statistically different morphometric differences between habitats are seen in the length of caudal peduncle which may represent local adaptations to differing flow regimes. Henault and Fortin (1989) also observed noticeable differences associated with caudal peduncle in spring and fall spawners of corigonids. They attributed this to higher growth rate in spring ciscoes, without studying the growth of the fish. The longer dorsal and paired fins in fishes collected from habitat I

can be related to the slow precise manoeuvring of the fish in the relatively stable lentic water body. Clarke (1973) reported an increase in surface area of the inhabiting water body would result in larger head. In *R. dandia* the length of head is more for those inhabiting lentic environments than for those fromlotic water bodies. No other interferences relating to head measurements could be reached from the present study.

The intraspecific differences in morphometric characters noticed in this study are likely to be primarily ecophenotype or a consequence of local response to dissimilar environments as Jerry and Cairns (1998) has reported for Australian bass inhabiting principally the freshwater reaches of river systems. Genetic polymorphism or environmentalfactors may induce morphological variability among spatially separated fishpopulations (Carvalho, 1993).The shape and structures are unique to the Serranid fishes from and the variations in its feature are probably related to the habit and habitat among the variants of this species(Cavalcanti *et al*., 1999).

Markert *et al.* (2010)examined genetic and morphological divergence among populations of two narrowly endemic cichlid species, *Teleogramma depressum* and *Lamprologus tigripictilis.* In *L. tigripictilis*, the strongest genetic break was concordant with measurable phenotypic divergence but no morphological disjunction was detected for *T. depressum* despite significant differentiation at mtDNA and nDNA microsatellite markers.

To test for genetic differentiation among fish inhabiting varied environments, the eye lens protein polymorphism has been used by the application of electrophoretic techniques. The same banding characteristics, their number of fractions, their concentrations and relative mobility for protein were similar in all the three gels. Four bands could be discernable in all the three and the Rm values of these were also the same. Thus the observed absence of any clear differences in the banding pattern reveals a large amount of inter- area homogeneity. Though substantial inter- area differences in morphometric characters could be detected among members of the species, there seems no genetic distinction between inhabitants of varied habitats. Pacific sardines from five widely separated localities are found to have little genetic variation both within and between populations (Hedgecock *et al*., 1989).

As the population admixture cannot occur in the areas selected, the observed pattern of genetic similarity in the species may be regarded as stable. Hence the degree of morphometric diversity among the members of different habitats can be attributed only to environmental differentiation.

REFERENCES

Austin, H.M, Scoles, D. and Abell,A.J. 1999. Morphometric separation of annual cohorts within mid– Atlantic bluefish, *Pomatomus saltatrix*, using discriminant function analysis. *Fish. Bull*., 97:411-420.

Bookstein, F.L. 1991. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge Univ. Press, New York.

Cavalcanti, M. J., Monteiro, L.R. and Duarte Lopes, P.R.. 1999. Landmark based morphometric analysis in selected species of Serranid fishes (Perciformes: Teleostei). *Zool. Stud*. 38:287–294.

Carvalho, G.R. 1993. Evolutionary aspects of fish distribution: genetic variability and adaptation. *J . Fish Bio.,* 43: 53-73.

Clabaut, C., Bunje, P.M.E, Salzburger,W. and Meyer, A. 2005.Geometric morphometric analysis provide evidence for the adaptive character of the Tanganyikan Cichlid fish radiations. *Evolution* , March 2007.

Dryden, I. L., and Mardia, K.V. 1998. *Statistical Shape Analysis*. John Wileyand Sons, New York.

Dube,K. and Dubey,G.P. 1987.Biometric studies of Indian mahseer *Tor tor* (Ham) from Narmada river. *Matsya*, 13:126-132.

Dutta, S., and Rao,B.V.S. 1981.Biometric comparison of samples of the clupeid fish *Escualosa thoracata* (Val.) from two localities. M*atsya*, 7: 50-63.

Elmer, K. R., Kusche H., Lehtonen , T.K and Meyer, A. 2010. Local variation and parallel evolution:morphological and genetic diversity across a species complex of neotropical crater lake cichlid fishes. *Phil. Trans. R. Soc. B*., 365: 1763–1782.

Ferguson, A. 1980.*Biochemical Systemetics and Evolution*. Blackie,Glasgow.

Gatz, A.J. 1979. Ecological morphology of freshwater stream fishes. *Ecology*, 60(4): 711-718.

Hedgecock, D.,Elmarie,S.H. Li, G., Sly, F.I. and Nelson,K. 1989. Genetic and morphometric variations in the Pacific Sardine, *Sardinops sagax caerulae*: Comparisons and contrasts with historical data and with variability in northern Anchovy, *Engraulis mordax. Fish. Bull*., 87(3): 653-671

Henault, M. and Fortin,R. 1989.Comparison of meristic and morphometric characters among spring and fall spawning ecotypes of cisco (*Coregonus artedii*) in Southern Quebec, Canada. *Can. J. Fish. Aquat. Sci*., 46: 166-173.

Hulsey, C.D. and Wainwright, P.C. 2002. Projecting mechanics into morphospace: disparity in the feeding system of labrid fishes. *Proc. Roy. Soc. London B, Biol. Sci.,* 269: 317-326.

Jerry, D.R. and CairnsS.C. 1998. Morphological variation in the catadromous Australian bass, from seven geographically distinct riverine drainages. *J. FishBiol*., 52(4): 829-843.

Karakousis, V., Triantaphyllidis, C.and Economidis,P. 1991.Morphological variability among seven Greek populations of brown trout (*Salmo trutta*). *J. Fish Biol*., 38:807-817.

Klingenberg C.P. and Ekau W. 1996.A combined morphometric and phylogenetic analysis of an ecomorphological trend: pelagization in Antarctic fishes (Perciformes: Nototheniidae). *Biol. J. Linn. Soc.,* 59: 143– 177.

Klingenberg, C.P., BarluengaM. and Meyer,A. 2003. Body shape variation in cichlid fishes of the *Amphilophus citrinellus* species complex. *Biol. J. Linn. Soc.,*80: 397–408.

Lammlei, U.K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature*, 227:680-685.

Markert,J.A., R.C Schelly and M.L.J. Stiassny 2010.Genetic isolation and morphological divergence mediated by high-energy rapids in two cichlid genera from the lower Congo rapids. *BMC Evolutionary Biology* 2010, 10: 149

Martin, E.R. and Pagare, S.D. 2012. Comparative electrophoretic studies of lens protein isolated from *puntiusticto* (Hamilton 1822) and *Rasbora daniconius* (Hamilton 1822). *The Bioscan*, 7(4): 571-574.

Piska, R.S. 1990.Racial analysis of carp minnows, *Salmo stomaclupeoides* (Bloch) *Indian J. Fish*., 37: 265-268.

Rüber, L. and Adams, D.C. 2001.Evolutionary convergence of body shape and trophic morphology in cichlids from Lake Tanganyika. *J. Evol. Biol.,* 14: 325–332.

Schluter D. 1993. Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. *Ecology,*74**:** 699–709.

Snedecor, G.W. and Cochran,K.G. 1975. *Statistical Methods*. Oxford and IBH Pub. Co., New Delhi.

Suneetha, G.K.B.2007. Morphological heterogeneity and population differentiation in the green chromid *Etroplus suratensis* (Pisces: Cichlidae) in Sri Lanka. *Ruhuna Journal ofScience*,2: 70-81.

Suneetha, G.K.B. and Damayanthi, H.G.B.N. 2008. Morphometric and isozyme confirmation for species level divergence between *Puntius dorsalis* (Pisces: Cyprinidae) and its presumed red-fin variety in Sri Lanka. *Ruhuna Journal of Science,* 3: 25-34.

Suvarna Devi, S. 1999. Inflence of habitat on the biology of *Rasbora daniconius* (Hamilton). Ph D thesis, Univ. Kerala.

Thorpe, R.S. 1987. Geographic variation: a synthesis of cause, data pattern and congruence in relation to subspecies, multivariate analysis and phylogenesis. *Bollettino di Zoologia*, 54:2-11

Turan, C., Erguden, D., Turan, F. and Gurlek, M. 2004. Genetic and morphological structure of *Liza abu* (Heckel, 1843) populations from the rivers Orontes, Euphrates and Tigris. *Turk. J. Vet. Anim. Sci.,* 28: 729-734.

Walker, J.A. 1997**.** Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol. J. Linn. Soc.,* 61**:** 3–50.