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Assessment of genetic variation in *Schizothorax Esocinus* Heckel, 1838 from Dal and Manasbal lakes of Kashmir

NAFATH-UL-ARAB[1], IRFAN AHMAD[1]✉, TASADUQ H SHAH[1], FAROOZ A BHAT[1], BILAL A BHAT[1], ZARKA YOUSUF[1], ASIM IQBAL BAZAZ[1] and NAKEER RAZAK[1]

Sher-e-Kashmir University of Agricultural Sciences and Technology, Rangil, Ganderbal, Jammu and Kashmir 191 201 India

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ABSTRACT

Schizothorax esocinus (Churru snow trout) Heckel, 1838 is a key freshwater species in the valley of Kashmir. The present study aimed to evaluate the genetic variability in the fish from the two eminent lakes of Kashmir valley namely Dal and Manasbal lakes, having different trophic gradients using biochemical (genetic) marker (SDS-Page). The electrophoretic analysis revealed 6 bands of molecular weights ranging from 13 to 150 kDa for Dal lake and 8 bands of molecular weights ranging from 15 to 150 kDa for Manasbal lake. The Rf value ranged from 0.15321 to 0.70625 for sampled fishes of Dal lake and from 0.14231 to 0.63559 for sampled fishes of Manasbal lake. The study provided some basic information about the genetic variation of *S. esocinus* populations in the Dal and Manasbal lakes. Electropherogram studies revealed that the studied populations of *Schizothorax esocinus* show some degree of variation in the electrophoretic migration of muscle proteins. Polymorphism in muscle protein is clearly demonstrated among this group of fishes from the two lakes.

Keywords: Genetic markers, Molecular weight, Protein, Rf, *Schizothorax*, SDS-Page

The first recognizable and the distinctive expression of genetic information is the protein. If two or more discontinuous forms of proteins occur in a species in such a proportion that the rarest protein cannot be maintained by recurrent mutation, it is known as protein polymorphism. Proteins are useful in studying the genetic variations within and among organisms. In order to assay polymorphism in fish species, three types of protein based techniques have been used namely serological methods, total protein analyses, and locus specific allozyme indicator (Cunniff 1998). Protein variations used for comparison of species dates back to 1906, when Nuttal used immunological methods to compare serum of human therewith of other primates. Genetic variability is directly assessed through molecular, allozyme, RAPD, RFLP, mtDNA, minisatellite and microsatellite markers. One of the conventional methods for characterizing fish stocks has been the comparative examination of morphological characters of the fishes (Hubbs and Lagler 1947). However, the traditional morphometric measurements are usually inefficient and biased, as they can produce an uneven coverage of the body form. Electrophoresis of proteins and histochemical staining methods (Hunter and Markert 1957, Smithies 1995) gained dominance over morphological studies during the mid 50's, by providing expeditiously possessed genetic figures. Proteins are considered as gene products and electrophoretic mobility's of various proteins in closely linked species or in diverse populations can be explained genetically (Bye and Ponniah 1983). Different electrophoretic techniques are used to recognize the variations among fish species and muscle protein is most usually used to assess the polymorphism among fish species (Smith 1990, Rashed *et al*. 2007, Haniffa *et al*. 2017). Intra-specific muscle protein variation in marine fish species was reported for the first time in *Anoplomoma fimbria* (Tsuyuki *et al*. 1965). The in-depth study on intraspecific polymorphism was done by Slecttitova *et al*. (1992) in European white fish and peled fishes of family coregonidea that showed polymorphism as well. Variability in species is present in response to survive and successfully react to the environmental changes (Ryman *et al*. 1995). An attribute of genetic variations in species is that it enhances the capability of an individual to adjust to the changing environment and these variations are necessary for survival of the species. Keeping in view the impediments in traditional genetic techniques and lack of sufficient study on the subject in the state of Jammu and Kashmir, the present study was undertaken.

Present address: [1]Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences and Technology, Rangil, Ganderbal, Jammu and Kashmir. ✉Corresponding author email: ahmadirfan@skuastkashmir.ac.in

MATERIALS AND METHODS

The present investigation on *Schizothorax esocinus* was carried out in Fish Genetics and Biotechnology Laboratory, Faculty of Fisheries, SKUAST-Kashmir.

Selection of sampling sites: For the study, 2 sites were selected. Fish samples were collected from the two famous lakes of Kashmir valley having different trophic gradients namely Dal lake, which is located in district Srinagar and Manasbal lake, located in Safapora, district Ganderbal. Sampling was carried out from two sites of Dal lake and two sites of Manasbal lake.

Collection of fish samples: Schizothorax esocinus, the experimental fish, was collected from two different lakes of Kashmir valley, viz. Manasbal lake and Dal lake. A total of 180 samples ranging from 140 g to 276 g in weight from Manasbal and 134 g to 250 g in weight from Dal lake were collected.

Protein extraction: Muscle was dissected from each *Schizothorax esocinus* specimen for analysis. About 1 g of the skeletal muscle tissue was dissected out from each sample and weighed. The extraction of protein was done by Tricholroacetic acid-acetone extraction method given by Ceruso *et al*. (2015).

Electrophoresis: Supernatant from tissues was analyzed using polyacrylamide gel electrophoresis (PAGE). Electrophoresis was carried out in a vertical gel apparatus according to the method given by Patel (1994), Gallagher (2000) and Laemmli (1970).

Staining of proteins: The staining of the protein was done by Coomassie brilliant blue stain by the method given by Laemmli (1970). Then photograph of gel was taken for further analysis with the help of gel documentation unit.

Statistical analysis: The statistical analysis was done using Ms-Excel and Paleontological Statistics Software Package (PAST).

RESULTS AND DISCUSSION

Comparative picture of the muscle protein profile of *Schizothorax esocinus* from Dal lake and Manasbal lake is presented in Fig. 1. The number of protein fractions of the muscle of *S. esocinus* under study from Dal lake resolved in 6 fractions whereas for Manasbal lake it resolved in 8 fractions. Maximum number of bands (8) was found in *S. esocinus* of Manasbal lake and minimimn (6) in Manasbal lake. The relative frequency (Rf) value of protein fraction was calculated according to the following formula:

$$\text{Relative frequency (Rf)} = \frac{\text{Distance travelled by the fraction}}{\text{Total distance travelled by the marker dye}}$$

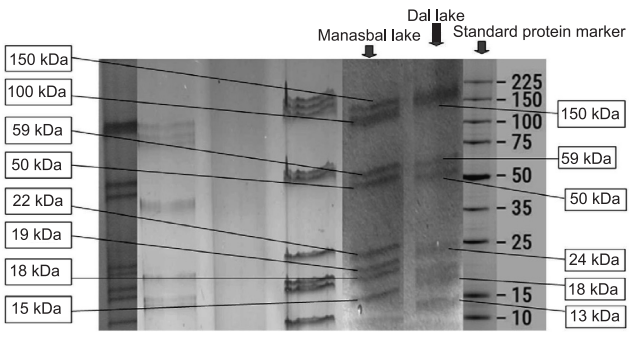

Fig 1. SDS-PAGE of muscle proteins of *Schizothorax esocinus* showing the band patterns from the two sampling sites.

For muscle of *S. esocinus*, the Rf value ranged from 0.15321 to 0.70625 for sampled fishes of Dal lake and from 0.14231 to 0.63559 for sampled fishes of Manasbal lake. A linear relationship ($y = -0.230x + 4.406$) was obtained when the logarithm of the molecular mass of the standard protein markers was plotted against their respective migrated distances by the band with value of $R^2=0.98$. The electrophoretic patterns were identical for all individuals belonging to the same population, regardless of the period of capture, sex, body weight or size of the fish. Velamala *et al*. (2017) studied genetic relationship of Snappers (Family: Lutjanidae) from Indian waters using SDS-PAGE. The study revealed that in *Lutjanus argentimaculatus*, the Rf values ranged from 0.014 to 0.959. In *L. fulviflamma*, the Rf values ranged from 0.014 to 0.893. The Rf values ranged from 0.014 to 0.893 for *L. fulvus*. In *L. johnii*, the Rf values ranged from 0.130 to 0.893. In *L. lemniscatus,* the Rf values ranged from 0.014 to 0.893. In *L. lutjanus,* the Rf values ranged from 0.014 to 0.893.

The molecular masses of the unknown proteins in *Schizothorax esocinus* from the two sites is given in Table 1. The range of the molecular masses of the unknown proteins of *Schizothorax esocinus* from Dal lake ranged from 13 kDa to 150 kDa whereas from Manasbal it was between 15 kDa to 150 kDa.

The sampled *Schizothorax esocinus* populations from the Dal lake in the study showed 4 common bands on the gel with the sampled *Schizothorax esocinus* populations of the Manasbal lake. The common bands had molecular weights of 150 kDa, 59 kDa, 50 kDa and 18 kDa. Two polymorphic protein bands were seen in the samples of the Dal lake on 5th and 10th fraction and had molecular weights of 24 kDa and 13 kDa whereas four polymorphic protein bands were seen on the gel from the Manasbal lake on 2nd, 5th, 7th and 9th fraction having molecular weights of

Table 1. Molecular weight (Da) of protein bands in electrophoretogram of *Schizothorax esocinus* from Dal and Manasbal lakes

Fraction number/ Band number	Dal lake (MW)	Manasbal lake (MW)
1	150,980.90	150,197.90
2	–	100,124.40
3	59,221.85	59,530.56
4	50,933.09	50,933.09
5	24,725.10	–
6	–	22,167.67
7	–	18,966.19
8	18,008.28	18,005.28
9	–	15,404.94
10	13,180.14	–
Total fractions	6	8

100 kDa, 22 kDa, 18 kDa and 15 kDa when visualized against the standard marker. In the present study, comparative picture of the muscle protein profile of *Schizothorax esocinus* from Dal lake and Manasbal lake revealed the number of protein fractions of the muscle of *S. esocinus*. The number of protein fractions in the present study was higher than the number of fractions reported in the same fish by Ganai *et al.* (2014). They studied the coupled biochemical genetic and karyomorphological analyses for taxonomic classification and found that the electrophoretograms of serum protein band patterns of *Schizothorax* species revealed similarities as well as differences in the number and molecular weight of protein band. *S. esocinus* showed 5 bands, *S. curvifrons* 5, *S. niger* 7, *S. labiatus* and *S. plagiostomus* each showed 6 bands. The present study is in agreement to the study conducted by Gul (2017) who reported that the protein bands in *Schizothorax niger* populations were different from the two locations. Gul (2017) also found that the protein bands in the *Schizothorax niger* samples of Dal lake resolved in three fractions and of river Jehlum resolved in four fractions. In the present study, the range of molecular masses of the unknown proteins of from Dal lake was 13 kDa to 150 kDa whereas the molecular masses of the unknown proteins from Manasbal was between 15 kDa to 150 kDa. The range of the molecular weight of the fish under investigation in the present study was different from the range of the molecular weight for the same fish species reported by Ganai *et al.* (2014). Ganai *et al.* (2014) found the range of molecular weight between 52 kDa to 121 kDa for *Schizothorax esocinus,* 41 kDa to 121 kDa for *Schizothorax niger,* 45 kDa to 121 kDa for *Schizothorax curvifrons,* 41 kDa to 121 kDa for *Schizothorax labiatus* and between 120 kDa to 45 kDa for *Schizothorax plagiostomus*. Similar studies were made by Habeeb and Mahdi (2013) to study the comparative electrophoretic studies of muscle proteins for two species of Tilapia. They found that the molecular masses of the bands resolved on the gel were between 18 to 123 kDa for *Tilapia zillii* and between 16 kDa to 73 kDa for *Oreochromis aureus* in freshwater fishes of Iraq. Jesslin *et al.* (2013) found the molecular weights of the protein bands to be 90, 65, 50, 40, 25 and 10 kDa in *Puntius bimaculatus;* 90, 80, 75, 65, 25, 20, 15 and 5 kDa in *Puntius filamentosus* and 80, 70, 60, 30, 24, 15 and 5 kDa in *Puntius tambraparniei.*

Four thick, broad and darkly stained protein bands were found at 150 kD, 24 kD, 18 kD and 13 kD from the samples of Dal lake and indicated an abundance of molecules in those bands, compared to the two faint or narrow bands at 59 kD and 50 kD. Similarly, 1 thick, broad, darkly stained protein band at 100 kD from the samples of Manasbal lake indicated an abundance of molecules in that band compared to the other 7 faint and narrow bands at 150 kD, 50 kD, 60 kD, 22 kD, 19 kD, 18 kD and 15 kD. In the present study, 4 thick, broad and darkly stained protein bands were found from the samples of Dal lake compared to the 2 faint or narrow bands. Similarly, 1 thick, broad, darkly stained protein band was found from the samples of Manasbal lake compared to the other 7 faint and narrow bands. Chakraborty (1990) studied the muscle and eye lens proteins of three species. A comparison of the electrophoretic pattern revealed that, apart from difference in the number and mobility pattern of each and every protein fraction, staining intensity and thickness of bands also differed. Habeeb and Mahdi (2013) found that the differences in thickness of protein bands for *T. zillii* and *O. aureus* in the invstigated areas and concluded that the difference in thickness of the bands may be due to difference in food items which were found in respective areas. Niolsen (2004) further concluded that thick, broad and darkly stained bands indicated an abundance of molecules in those bands.

The sampled *Schizothorax esocinus* populations from the Dal lake in the present study showed 4 common bands on the gel with the sampled *Schizothorax esocinus* populations of the Manasbal lake. Two polymorphic protein bands were seen in the samples of the Dal lake on 5th and 10th fraction whereas four polymorphic protein bands were seen on the gel from the Manasbal lake on 2nd, 5th, 7th and 9th fraction when visualized against the standard marker. The present study is in concurrence with the studies made by Demir *et al.* (2011), who found that there were common as well as polymorphic protein bands found in four populations of *Salmo trutta macrostigma*. Abu-Almaaty *et al.* (2017) studied the genetic variability of three fish species of genus *Puntius*. The protein analysis by SDS-Page produced 29 bands, 21 common bands and 7 polymorphic bands and one unique band in all three species. However, variations between the individuals of one species, as exemplified by the results on codling (Connell 1953) are small, and it can reasonably be assumed that the variations which occur with other species are also small. Samad (2003) concluded that variations in protein patterns from two different environments could be used as a marker to identify two separate breeding populations of the fish.

The electrophoretic patterns in the present study (Fig. 2) were identical for all individuals belonging to the same population, regardless of the period of capture, sex, body weight or size of the fish and the present study is in agreement to the studies made by Tranvouez and Rodoni (1990) who found identical electrophoretic patterns for all individuals belonging to the same population of Cephalopods, regardless of the period of capture, sex, body weight or size. Bulbul and Kutrup (2007) also found that all the specimens of skeletal muscles of *Bufo viridis* used in the SDS-PAGE experiments did not show any differences between males and females.

There is a reduction in the genetic resources of natural fish populations due to over utilization of fish stocks, pollution and human intervention. Conservation of this fragile genetic diversity is extremely important to maintain ecological balance. The individuals with greater genetic variability perform better (growth), are viable, highly fecund and resistant to environmental stress. Genetically variant species have proven valuable for aquaculture and fisheries management, identification of stocks, breeding programme,

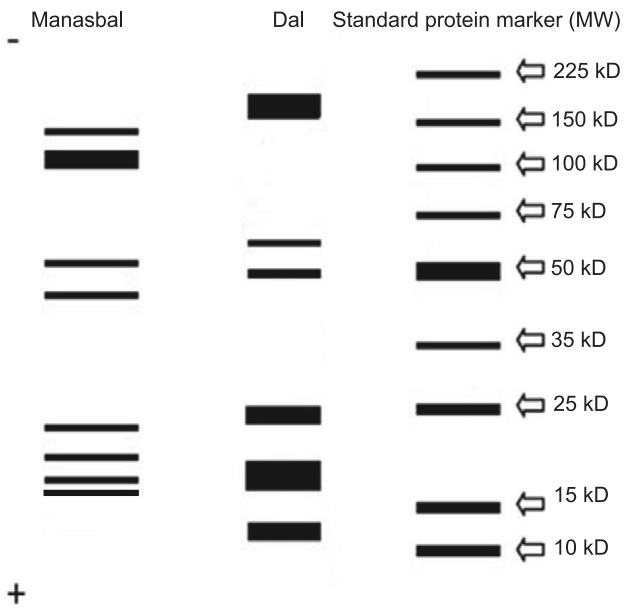

Fig. 2. Zymogram of the electrophoretic pattern in the muscle protein of *Schizothorax esocinus* from Dal and Manasbal lakes.

restoration of ecology and estimation of genetic contributions in stock. Apart from this, consolidated approaches from grass root level to administrative level must be consummated for managing the *Schizothorax* species in the Kashmir valley.

Thus, the approach of this study with muscle protein can be very useful in seeking and revealing genetically based biochemical polymorphisms. It will enable aquaculturists to recognize or identify the right species to be used in breeding for accurate results and profitability after employing basic and molecular methods. In conclusion, the present study indicated that the studied populations of *Schizothorax esocinus* show some degree of variation in the electrophoretic migration of muscle proteins. Polymorphism in muscle protein is clearly demonstrated among this group of fishes. However, a detailed study involving the morphometric, environmental and molecular aspects may further confirm the present findings explicitly. Further studies are recommended on determining the other possible variations in this species from other lakes of the valley. The present study suggests that the genetic variations in *S. esocinus* should be considered as one of the components in breeding programmes and in aquaculture and fisheries management. It also suggests that brooders from both the lakes should be introduced during the breeding programme of this fish in order to have better performing fish populations.

REFERENCES

Abu-Almaaty A H, Bahgat I M and Al-Tahr Z M. 2017. Genetic variability of three fish species of genus *Puntius. Journal of Experimental and Applied Animal Sciences* **2**(2)*:* 153–64.

Bulbul U and Kutrup B. 2007. Comparison of skeletal muscle protein bands among five populations of *Bufo virdis* in Turkey by SDS-PAGE. *Turkish Journal of Zoology* **31**: 419–22.

Bye V J and Ponniah A G. 1983. *Application of Genetics in Aquaculture*. CMFRI Spl. Publication, **13:** 90.

Ceruso M, Chirollo C, Boccia F, Smaldone G, Marrone R and Pepe T. 2015. Evaluation and comparison of four protein extraction protocols for mono- and two- dimensional electrophoresis in *Mytilus galloprovincialis*. *Italian Journal of Food Safety* **4:** 4534.

Chakraborty S K. 1990. Electrophoretic study on muscle and eye lens proteins of three Sciaenids. *Indian Journal of Fisheries* **37**(2): 93 – 98.

Connell J J. 1953. Studies on the proteins of fish skeletal muscle. *Biochemistry Journal* **54**: 119.

Cunniff P A. 1998. *Ofûcial Methods of Analysis of AOAC International*. Vol. 1, 16th edn. 4th Revision. *Association of Ofûcial Analytical Chemists*, Washington, D.C. 2110 pp.

Demir O, Günlü A, Küçük F, Gülle S and Gümü E. 2011. Analysis of sarcoplamic proteins in natural populations of mountain trout (*Salmo trutta macrostigma* Dumeril, 1858) with SDS-PAGE. *African Journal of Biotechnology* **10**(55): 11758–63.

Gallagher S R. 2000. *Current Protocols in Protein Science*. (Eds) Coligan J E, Dunn B M, Ploegh H L, Speicher D W and Wingfield P T. pp. 10.1.29–10.1.34. Jhon Wiley & Sons, New York.

Ganai F A, Wani S, Ahmad S, Yousuf A R and Tripathi N K. 2014. Coupled biochemical genetic and karyomorphological analyses for taxonomic classification- A case study of *Schizothorax* species complex (Teleostei: Cyprinidae). *African Journal of Biotechnology* **13**(15): 1623–30.

Gul S. 2018. 'Study of morphological differentiation between riverine (Jhelum) and lacustrine (Dal) populations of snow trout *Schizothorax niger*.' M.F.Sc thesis, Faculty of Fisheries, SKUAST-Kashmir.

Habeeb F S and Mahdi A A. 2013. Comparative electrophoretic studies of muscles proteins for two species *Tilapia zillii* and *Orochromis aureus* in fresh water fishes of Iraq. *Basrah Journal of Agriculture Science* **26**(2): 258–71.

Haniffa M A, Jeya S P, Milton J M and Kavitha K. 2017. Protein profiling for phylogenetic relationship in snakehead species. *Iranian Journal of Fishery Sciences* **16**(4): 1178–86.

Heckel J J. 1838. Fische aus Caschmir gesammelt und herausgegeben von Carl Freiherrn von Hügel, beschrieben von J.J Heckel. Wien. 1–86, 1–12.

Hubbs C L and Lagler K F. 1947. Fishes of the Great lakes region. Cranbrook Institute of Scrience. *Bulletin* **26:** 186.

Hunter R L and Markert C L. 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. *Science* **125:** 1294–95.

Jesslin G, Sabaridasan A, Edwinthangam P, Palanikani R and Soranam R. 2013. Electrophoretic studies on the muscle proteins of three species of genus *Puntius* (Osteichthyes - Cyprinidae). *International Journal of Research in Fisheries and Aquaculture* **3**(2): 16–20.

Laemmli U K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**(5259): 680–85.

Niolsen M I. 2004. Variation chemical in protein and amino acid of (Teoleostei: Sparidae) *Acanthopagrus australis*. *Journal of Fish Biology* **67**: 250–547.

Patel D. 1994. Gel electrophoresis. (Eds) Rickwood D and Hames B D. pp. 3–5. Jhon Wiley & Sons, New York.

Rashed M A, Tantawi T M A, Atta A H, Salah S H and El-Ganal A A. 2000. Plasma and muscle protein electrophoretic locality variations in catfish (*Clarias gariepinus* L.) *Eygyptian*

Journal of Genetics and Cytology **29**(2): 127–46.

Ryman N, Utter F and Laikre L. 1995. Protection of intraspecific biodiversity of exploited fishes. *Review of Fish Biology and Fisheries* **5**: 417–46.

Slecttitova V, Slechta and Valenta M. 1992. Biochemical markers of *coregonids* in Czechoslovakia. *Biology and Management of Coregonid Fishes.* Todd T N and M Lucynski (Eds). **39**(3–4): 563–70.

Smith P J. 1990. Protein electrophoresis for identification of Australian fish stocks. *Australian Journal of Marine and Freshwater Research* **41**: 823–33.

Smithies O. 1955. Zone electrophoresis in starch gels: group variation in the serum proteins of normal human adults. *Biochemical Genetics* **61**: 629–41.

Tranvouez Y and Rodoni R B. 1990. Eye lens SDS-page electrophoresis and the systematic of Cephalopods. *Cahiers de Biologie Marine* **31**: 281–89.

Tsuyuki H, Roberts E and Vanstone W E. 1965. Comparative zone electropherograms of muscle myogens and blood hemoglobins of marine and freshwater vertebrates and their application to biochemical systematics. *Journal of the Fisheries Research Board of Canada* **22**: 203–11.

Velamala G R, Babu K R and Naranji M K. 2017. Genetic relationship of Snappers (Family: Lutjanidae) from Indian waters using SDS-PAGE technique. *Genetics of Aquatic Organisms* **1**: 49–55.