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Full Length Research Paper

Analysis of sarcoplamic proteins in natural populations of mountain trout (*Salmo trutta macrostigma* Dumeril, 1858) with SDS-PAGE

Orhan Demir[1], Ali Günlü*[1], Fahrettin Küçük[1], İskender Gülle[2] and Erkan Gümüş[3]

[1]Fisheries Faculty, Süleyman Demirel University, Eğirdir-Isparta, Türkiye.
[2]Science and Arts Faculty, Mehmet Akif Ersoy University, Burdur, Türkiye.
[3]Fisheries Faculty, Akdeniz University, 07058, Antalya, Türkiye.

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Molecular weights and band counts of sarcoplasmic proteins in four populations of S*almo trutta macrostigma* living in Mediterranean region of Turkey were analyzed with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method and densitometric analysis. 13, 17, 14 and 15 sarcoplasmic protein bands were obtained, respectively from Eşen Stream (I), Aksu Stream (II), Alara Stream (III) and Alakır Stream (IV) populations. Protein bands with molecular weights 210, 93 and 30 kDa were found solely in population II. 116 kDa bands were present in all populations but population I with density of bands with molecular weights over 97 kDa was smaller than all the other populations. Densitometric analysis of population IV showed that it differs from the three other poulations by having all protein bands except for 45 kDa band which were at low densities.

Key words: Sarcoplazmic proteins, mountain trout, *Salmo trutta macrostigma*, SDS-PAGE, electrophoresis.

INTRODUCTION

Sarcoplasmic, myofibrillary and connective tissue proteins are found in fish muscle tissues (Love, 1997). Connective tissue proteins from 3 to10% of the muscle proteins depending on species, nutritional regime and maturation (Huss, 1995; Love, 1997). Myofibrillary proteins (myosin, actin, tropomyosin and actomyosin) constitute most of the fish muscle proteins (70 to 80%) (Huss, 1995). The remaining 25 to 35% is formed by sarcoplasmic proteins which include low molecular weight (40 to 60 kDa) proteins like myoalbumin, globulins and enzymes that can be extracted from water and neutral salt solutions and separated by electrophoretic methods (Huss, 1995; Love, 1997).

Structure and features of muscle proteins are affected by physiological factors, environment, seasons, stress, starvation, breeding season and migration (Ando et al., 1985; Ando et al., 1986; Gomez et al., 2000; Ladrat et al., 2000; Delbarre-Ladrat et al., 2006). In a previous study, differences in breeding season and some reproductive features between mountain trout populations were revealed (Demir et al., 2010).

Alongside classical morphology, electrophoresis and isoelectric focusing methods (Huss, 1995; Love, 1997) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Chen and Hwang, 2002) are used in fish and crustacean taxonomy. It is also known that sarcoplasmic proteins, myoglobin, serum, glicolytic enzymes and liver proteins are used in such electrophoretic studies (An et al., 1988; Scobbie and Mackie, 1988; Yılmaz et al., 2005). Fish sarcoplasmic proteins are not denatured immediately after death unlike most other proteins (Kjærsgård and Jessen, 2003). Its low cost and feasibility provided electrophoretic analysis of sarcoplasmic proteins in a wide usage area in phylogeny and phylogeography studies (Scobbie and Mackie, 1988; Huss, 1995; Love, 1997; Chen and Hwang, 2002; Yılmaz et al., 2005). SDS-PAGE method was used to determine <30 kDa proteins that cause ciguatera in *Lutjanus bohar* (Chen et al., 2010).

Protein banding and molecular weights are used also in taxonomy of fishes (Khoo et al., 1997; Piñeiro et al.,

*Corresponding authors. E-mail: aligunlu@sdu.edu.tr. Tel: +90 2463133447. Fax: +90 2463133452.

1997; Knuutinen and Harjula, 1998; Colombo et al., 2000; Yılmaz and Ayaz, 2005; Yılmaz et al., 2005; Yılmaz et al., 2007). Presence of changes as results of some processing techniques in banding patterns and molecular weights in various animal products like fresh and processed beef and fish have been reported (LeBlanc and LeBlanc, 1989; Ragnarsson and Regenstein, 1989; LeBlanc and LeBlanc, 1994; Garcia et al., 1997; Türköz et al., 2000; Thorarinsdottir et al., 2002; Ekici and Akyüz, 2003; Hultmann et al., 2004). A relative increase was reported in myosin amount and total optical density in muscle extracts of common dentex (*Dentex dentex*) fed with low protein feeds (Suárez et al., 2009).

Mountain trout also known as Mediterranean or Anatolian trout (*Salmo trutta macrostigma* Dumeril 1858) is a freshwater fish taxon widely distributed in Turkish river systems (Alp and Kara, 2004; Kocaman et al., 2004), in clean, well oxygenated, fast flowing freshwater habitats in which water temperatures do not exceed 20°C and in altitudes between 50 to 2300 m (Gülle et al., 2007). Along with other migrating freshwater fish species, natural trout populations are declining or disappearing due to adverse effects to ecosystems such as splitting of breeding ranges by dam constructions, breeding habitat deterioration, subtle changes in water regimes and intense fishing pressure (Jonssonn et al., 1999; Marzona et al., 2003).

Presence of four different subspecies including *S. t. macrostigma* is a widely accepted argument about the taxonomy of inland *Salmo trutta* populations of Turkey. However, in his revison of genus *Salmo,* Kottelat (1997) stated that populations from Corsica, Sardinia, Sicily, Italy, Balkans and Turkey previously referred to *S. t. macrostigma* belonged to a separate taxon and true *S. t. macrostigma* is found only in Algeria. Turan et al. (2009) determined Çoruh population of *S. t. macrostigma* in NE Turkey as a new species (*Salmo rizeensis*) and *S. t. labrax* populations of Çoruh river, environs of Arhavi, İyidere and Fırtına streams as another new species (*S. coruhensis*). In the same study, they reported that *S. abanticus* is from Abant Lake and freshwaters around Bolu*, S. platycephalus* from upper tributaries of Seyhan river, *Salmo caspius* in Kura and Aras rivers and, with reference to revision of Kottelat (1997), all populations in the Mediterranean part Turkey as *S.* cf. *macrostigma*. Togan et al. (1995) reported that the genetic structure of two brown trout Salmo trutta populations living in Lake Abant in Bolu and Uzum River in Antalya were examined using starch gel electrophoresis. The two populations were found genetically different.

In such endemic rich regions like Anatolia where speciation can be observed within small geographical areas, morphological methods are not sufficient to solve taxonomical problems. Besides, electrophoresis has become an important method in taxonomy and characterization of food by the analysis of proteins.

In this study, four different populations of taxonomically problematic mountain trout (*S.t. macrostigma*) living in the Mediterranean part of Turkey whose positions are still under discussion were examined. Weights and protein bands of sarcoplasmic proteins in each population were determined using SDS-PAGE and densitometry. Also, similarity and distance between populations were investigated.

MATERIALS AND METHODS

Mountain trout specimens were caught in April 2008 from Eşen (I), Aksu (Köprüçay) (II), Alara (III) and Alakır (IV) streams in SW Turkey (Figure 1). Demir et al. (2010) reported that some physical and chemical parameters of water in the four localities are similar. Two specimens from each locality were used for the analyses. Average weights of specimens were found as 96.49 ± 2.20, 69.38 ± 23.99, 108.92 ± 18.74 and 59.59 ± 8.18g respectively. Specimens were carried in foam boxes filled with ice cubes and brought to the Food Laboratory in Süleyman Demirel University, Fisheries Faculty within 5 h. After weighing, internal organs of the specimens were cleaned out and filets were stored under -80°C till SDS PAGE analyses.

For protein extraction, 0.5 g of trout filets were weighed and put into 0.85% NaCl solution (at 1/20 rate) and homogenized at 16000 rpm for 3 min with a Heidolph Diax 900 (Germany). Then, the homogenized tissues were centrifuged in a Sigma 2 to 16 K cooling centrifuge (Germany) at 5000 rpm and 4°C for 15 min and the supernatant was used in total microprotein and SDS-PAGE analyses. Total microprotein analyses were done by using a Sigma TP0300 (Total protein kit, micro Lowry, Peterson's modification) (Lowry et al., 1951; Peterson, 1977).

SDS-PAGE analysis was done according to the method described by Laemmli (1970) in vertical electrophoresis device (Bio-Rad, Mini-Protean II Cell, and USA). The extract was centrifuged at 12500 rpm for 3 min (Sigma 2 to 16 K, Germany); 1 part of supernatant was then mixed with 3 parts of SDS-PAGE sample tampon solution. Samples prepared for SDS-PAGE were boiled at 95 °C for 4 min to complete protein denaturation. Slab gel was formed by 7.5% separating gel and 4% stacking gel. A lyophilized protein standard (Sigma Cat. No: S8445) was used to calculate molecular weights of the proteins. The standard consisted of: soybean trypsin inhibitor (20 kDa), bovine pancreas trypsinogen (24 kDa), bovine erythrocyte carbonic anhydrase (29 kDa), mouse muscle glyceraldehyde-3-phosphate dehydrogenase (36 kDa), chicken egg ovalbumin (45 kDa), bovine liver glutamic dehydrogenase (55 kDa), bovine serum albumin (66 kDa), mouse muscle β phosphorylase (97 kDa), *E. coli* β-galactosidase (116 kDa) and mouse muscle myosin (205 kDa). Sarcoplasmic proteins were run in SDS-PAGE at 20 to 35 mA for 3 to 4 h on average (Bio-Rad PowerPac 300, USA). After electrophoresis, the gels were stained with coomassie brillant blue (CBB) R-250 overnight. Afterwards, removal of the residual stain on the surface of gels was done with a destaining solution. Finally, gels preserved in 7% acetic acid were photographed on a white illuminated desk in a dark room. Molecular weights of the proteins were calculated according to Weber et al. (1972). Densitometric analysis of protein bands were done using ImageJ program v. 1.3 (Rasband, 1997).

RESULTS AND DISCUSSION

The results of this study showed that 13, 17, 14 and 15 muscle protein bands were found in populations I, II, III, and IV of *S. t. macrostigma,* respectively (Figure 2).

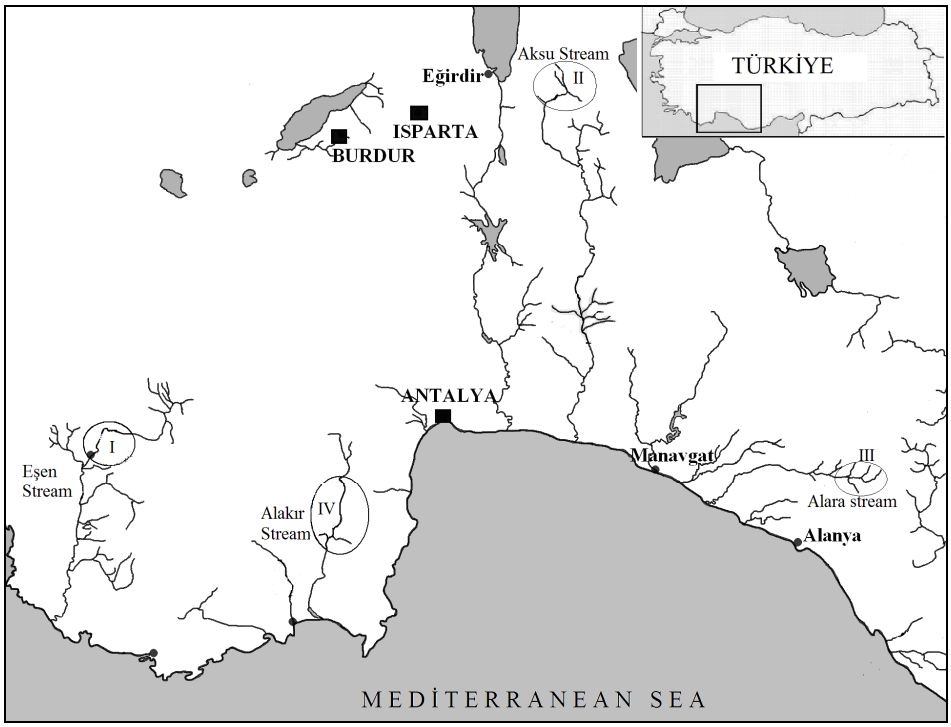

Figure 1. Natural mountain trout specimens were caught in locality (Eşen-I, Aksu-II, Alara-III, and Alakır-IV streams in SW Turkey).

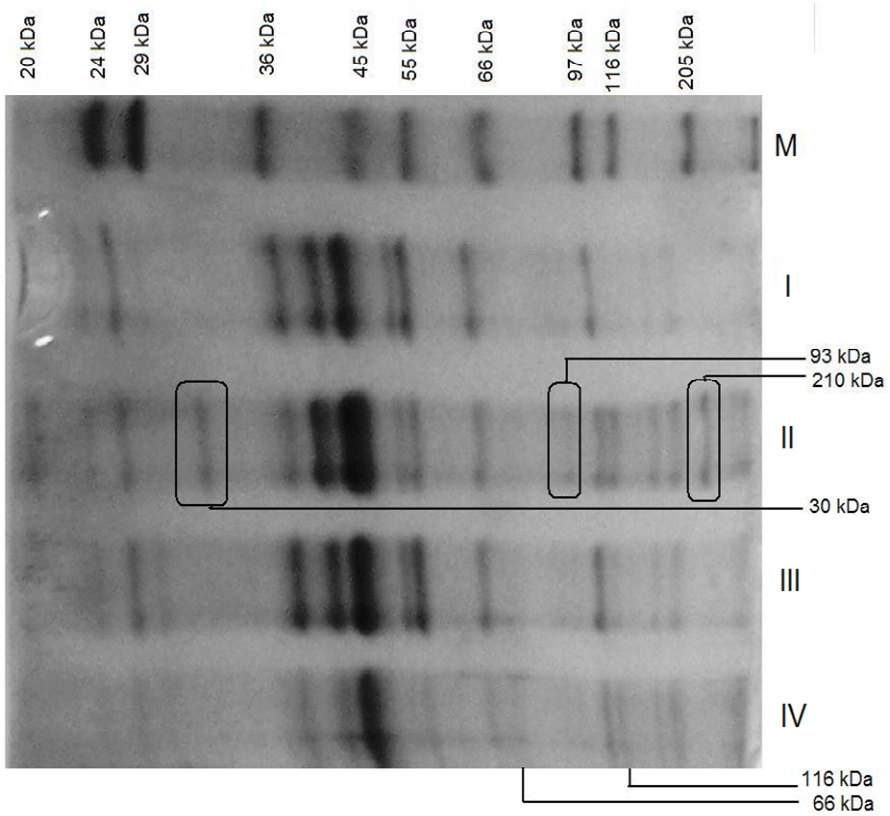

Figure 2. SDS PAGE electrophoresis of sarcoplasmic proteins from natural mountain trout populations. Eşen, I; Aksu, II; Alara, III; Alakır, IV.

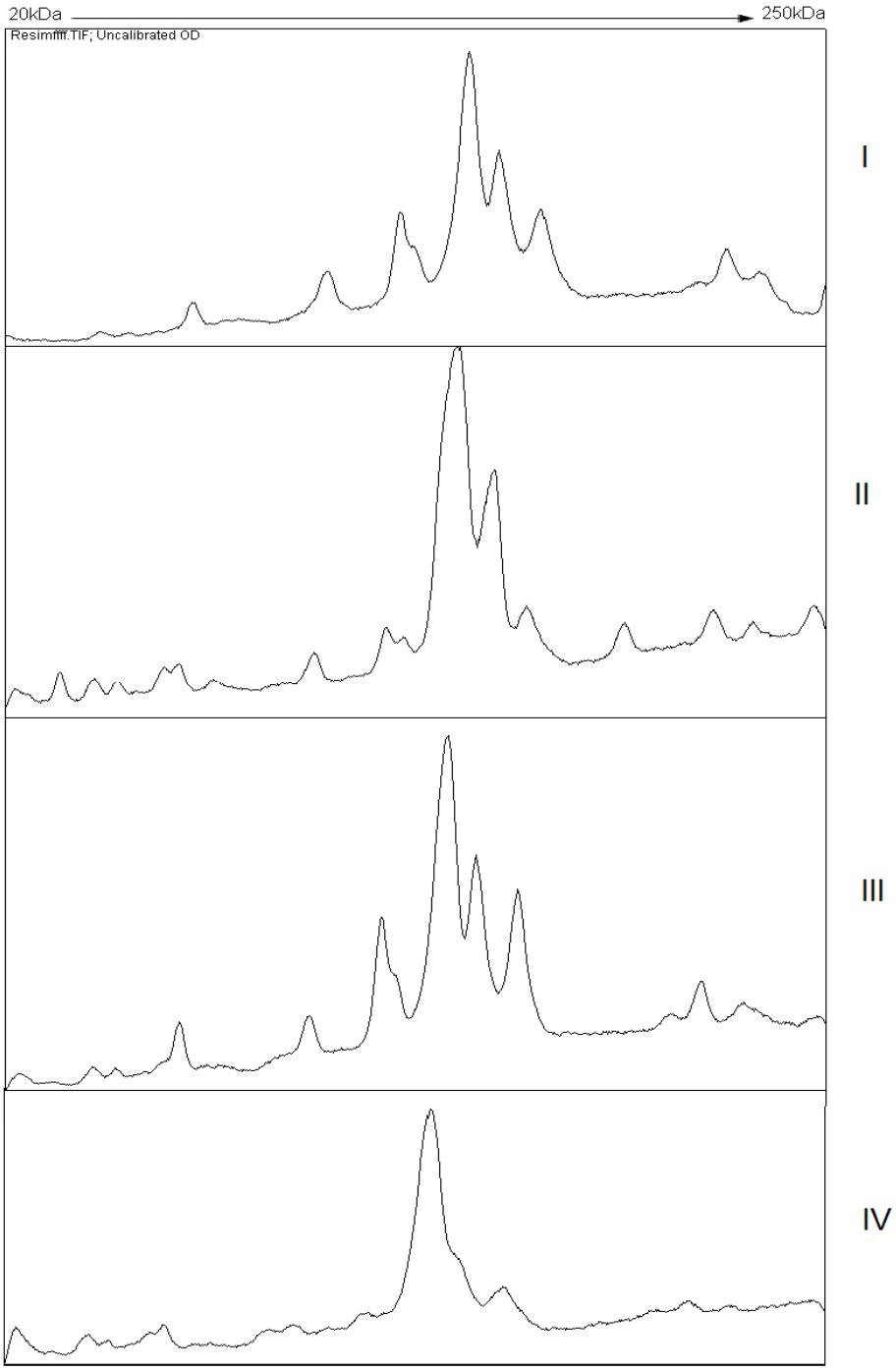

Figure 3. Densitometric analysis of sarcoplasmic proteins from natural mountain trout populations Eşen, I; Aksu, II; Alara, III; Alakır, IV.

Three bands with molecular weights of 210, 93 and 30 kDa were found solely in population II (Aksu). 116 kDa band was present in populations II, III and IV and 66 kDa band having conspicuously smaller density than other bands was found only in population IV (Figure 2). According to the densitometry analysis, the presence of bands with molecular weights over 97 kDa were found in population I, but the density of the bands were also smaller than those of all the other populations. It has also found that population IV differed from the other populations by having all protein bands except for 45 kDa band which were at low densities (Figures 2 and 3).

With reference to band numbers and molecular weights, presence of differences among the studied

natural populations of *S.t. macrostigma* in the Mediterranean part of Turkey was determined (Figures 2 and 3).

Previous studies indicate that these differences may be used in taxonomy: Knuutinen and Harjula (1998) found differences in band numbers and molecular weights among 16 freshwater fish species, Colombo et al. (2000) in 14 fish species, and Khoo et al. (1997) among four color variants of *Beta splendens*. However, Piñeiro et al. (2001) showed close relation among five different hake species with this method. Durna (2010) reported close similarity between protein bands of *Cyprinus carpio* and *Chalcalburnus chalcoides*.

The differences among the studied populations in band numbers and molecular weights may have been caused by factors given by Ladrat et al. (2000), Gomez Guilllen et al. (2000), Ando et al. (1986) and Ando et al. (1985). These structural differences were mirrored by some reproductive features (Demir et al., 2010).

In a study on muscle extracts from sea bass (*Dicentrarchus labrax*), Chéret et al. (2006) found that 200, 105, 42, 36 and 34 kDa protein bands belonged to myosin, α-actinin, actin, glyceraldehyde-3-phosphate dehydrogenase and tropomyosin, respectively, and 32 kDa band was to tropomiyosin degraded by B and L catepsine. 200 kDa band which was determined by Ladrat et al. (2003) and Chéret et al. (2006) as strong myosin chain in myofibrillary extract corresponds to the 210 kDa band in population II. The 51 and 54 kDa bands found to be present in all the populations should be desmin, which was determined as 49 and 53 kDa by several researchers (Verrez-Bagnis et al., 2001; Ladrat et al., 2003). Also represented in all populations, the broad 44 kDa band is thought to be the broad band stated by Ladrat et al. (2003) and Chéret et al. (2006) which resulted by overlap of 42 kDa actin band and keratin kinase and aldolase bands between 39 to 42 kDa. The 30 kDa protein band found uniquely in population II should be troponin T as stated by Ladrat et al. (2003) and Chéret et al. (2006).

Despite the diversity in mt-DNA sequences among 27 *Salmo trutta* populations from Turkey, Bardakçı et al. (2006) found a distinct relation between lineages and geographical distribution of populations. However, according to unweighted pair group method with arithmetic mean (UPGMA) tree in their study, Alakır and Köprüçay populations were not closely related. Similarly, the populations were found to be distinct according to sarcoplasmic protein bands. This difference is also mirrored by physical features and morphology of the populations as red spots were fewer and paar marks were more numerous in Alakır population than that of Aksu (upper basin of Köprüçay river) population.

In conclusion, some differences were found in band numbers and molecular weights among the populations of *S. t. macrostigma* living in the different river systems. Therefore, the need for more detailed work to clarify taxonomic relations of these populations was indicated by our results.

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