

Comparative analysis of protein profile of Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), and hybrid (F₁ *Acipenser baerii* x *Acipenser gueldenstaedtii*) grown on an aquaculture farm

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Abstract

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In the present study, an electrophoretic technique was used to determine protein profiles of sarcoplasmic, myofibrillar, and alkali-soluble proteins in sturgeon meat of different genotypes (Russian sturgeon, Siberian sturgeon and their F₁ hybrid). SDS-PAGE of the protein fractions showed that sarcoplasmic and myofibrillar proteins differed the most. High molecular weight polymers (>250 kDa) were observed in the myofibrillar fraction in each weight group of the three genotypes. The highest number of bands (20) was observed in the sarcoplasmic protein fraction of the Russian sturgeon. Low molecular weight fractions (<50 kDa) were dominant in each investigated genotype. In the studied hybrids, proteins with molecular weights which were not found in the studied individuals from the Russian and Siberian sturgeon, were registered. No such trend was observed in alkali-soluble proteins of the hybrids where all observed bands corresponded to the studied individuals Russian and Siberian sturgeon. The number of protein bands and their mobility (respectively molecular weight) is important for species characterization and identification.

Keywords: sturgeon; aquaculture; muscle proteins; sarcoplasmic, myofibrillar, alkali-soluble proteins

Abbreviations: SP – sarcoplasmic proteins; MP – myofibrillar proteins; ASP – alkali-soluble proteins; MW – molecular weight; LMW – proteins with molecular weights ≤ 50 kDa; MMW – proteins with molecular weights from 51 to 150 kDa; HMW – proteins with molecular weights over 150 kDa.

Introduction

The members of the family Acipenseridae are highly valued because of the delicacies which they produce. These relict fish are currently endangered. Cultivation of sturgeon in aquaculture farms is one of the most important tools for saving natural populations. Through this type of cultivation, the market is saturated with demanded products, reducing the anthropogenic pressure on endangered species in natural ecosystems.

In sturgeon breeding, both various species and hybrids are grown (Chebanov & Galich, 2013). Russian and Siberian sturgeon are commonly cultivated species. Russian sturgeon is an anadromous species inhabiting the basins of the Caspian, Black, and Azov Seas, and their adjacent large rivers. The Siberian sturgeon is a potamodromous species distributed in the reservoirs of Siberia. Ruban (2019) pointed out that the Siberian sturgeon is characterized by a very wide rate of reactions, allowing the species to adapt well to dif-

ferent thermal and trophic conditions outside its range. Due to these features, the Siberian sturgeon has become a preferred species in the production of meat and caviar (Bronzi et al., 2019; Chebanov & Williot, 2018). The species is often used for industrial hybridization (Miburo, 2018; Shivaramu et al., 2019). Hybridization is widely used in aquaculture as a method to increase production efficiency (Bartley et al., 2001).

Since 1998, international trade in all sturgeon products and parts has been regulated (CITES Notifications, No. 1998/13 Geneva, 31 March 1998). However, illegal fishing continues to threaten many natural populations. Illegal fishing and trade continue to be a threat to the destruction of these fish. To regulate legitimate trade and to prevent illicit trade, it is necessary to develop a single identification system for parts and products of Acipenseriformes. In addition, as more and more new species and hybrids enter sturgeon farming, the risk of genetic contamination of natural populations increases (Ludwig et al., 2009; Tsekov & Cekov, 2012). The problems with the identification of live fish and sturgeon products are becoming more and more important.

Gao et al. (2021) noted that in sturgeon more attention is paid to caviar, but excluding caviar, the use of sturgeon products is low and studies related to them are insufficient. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing have been successfully used to identify caviar from different sturgeon species (Chen et al., 1996; Al-Holy & Rasco, 2006). The electrophoresis was successfully used for the species identification of raw fish and to assess the freshness and falsification of products (Montowska & Pospiech, 2007).

Similar approaches and methods are used in the study of muscle proteins in warm-blooded animals and fish (Malva et al., 2018). SDS-PAGE is a technique widely used to separate proteins according to their electrophoretic mobility and this tool can be used in the classification of fish. The protein profile of meat has been studied in different species of fish: *Sardinops melanosticta* and *Pneumatophorus japonicus japonicus* (Hashimoto et al., 1979); *Sardinella gibbosa* and *Rastrelliger kanagurta* (Chaijan et al., 2004); some species of *Tetraodontidae* (Chen & Hwang, 2006). Concerning sturgeon species, the research mainly concerns the genetic and biochemical identification of species and hybrids in natural populations (Peycheva et al., 2004; Dobrovolev et al., 2005; Tsekov et al., 2008; Tsekov & Cekov, 2012).

The aim of the present study is a comparative characteristic of the protein profile of meat in Siberian sturgeon, Russian sturgeon, and their F₁ hybrid, cultivated in industrial aquaculture.

Materials and Methods

The study was conducted with male individuals of different genotypes:

- Siberian sturgeon (*Acipenser baerii*) – Ab;
- Russian sturgeon (*Acipenser gueldenstaedtii*) – Ag;
- hybrid (F₁ *Acipenser baerii* x *Acipenser gueldenstaedtii*) – Hy.

The fish were selected from commercial stocks at random. The fish were cultured in net cages farm, located in a dam in southeastern Bulgaria (with coordinates: 41°37' N latitude and 25°20' E longitude).

Fish of different species and categories were reared in separate cages on the farm. The cages were 8×8 m in size with a depth of 6 m below the water surface. Each cage was equipped with double polyamide nets. Feeding was performed with a commercial specialized sturgeon granular extruded mixture (Table 1).

Table 1. Composition of the commercial feed

Indices	Value
Protein, %	46.00
Fat, %	15.00
Crude fibre, %	1.40
Ash, %	6.50
Total P, %	1.03
Ca, %	1.40
Na, %	0.30
Vitamin A, IU.kg ⁻¹	10 000
Vitamin C, mg.kg ⁻¹	520
Vitamin E, mg.kg ⁻¹	200
Vitamin D ₃ , IU.kg ⁻¹	2303
Gross energy, MJ.kg ⁻¹	21.00
Digestible energy, MJ.kg ⁻¹	19.20

Throughout the rearing period, according to the rearing technology, the fish on the farm were sorted by live weight.

At the end of the vegetation period (November), five fish from each cage with a smaller (first weight group) and larger (second weight group) live weight were randomly taken from each genotype. In this way, two weight groups of each genotype were formed – 1 and 2 (Table 2).

In the slaughter analysis of the fish, homogenized samples were prepared from the muscles of the fillet (without skin) for further analysis.

Fractionation of fillet muscle proteins. The extraction process was performed as described by Bagthasingh et al. (2016) with some modifications: two grams of each sample were homogenized with 30 ml of 0.05 M phosphate buffer (pH 7.0), centrifuged for 20 min at 1800 × g (MPW-251,

Table 2. Characteristics of two weight groups of each genotype

Genotype	Weight group	Group code Genotype / weight group	n	Body weight*
Siberian sturgeon	1	Ab-1	5	2823.00±126.044 ^a
	2	Ab-2	5	4273.20±110.288 ^a
Russian sturgeon	1	Ag-1	5	3010.80±72.950 ^b
	2	Ag-2	5	4910.00±100.300 ^b
Hybrid	1	Hy-1	5	2796.60±129.839 ^c
	2	Hy-2	5	4934.80±192.539 ^c

a, b, c The values with the same letters in the rows are statistically different: ($p < 0.001$); *mean ± standard error of the mean

Med. Instruments, Warszawa, Poland) and the supernatants were collected, the protein was estimated and reported as sarcoplasmic proteins (SP). To the solid mass, 30 ml of 0.05 mM phosphate buffer (pH 7.0) containing 0.5 M potassium chloride was added, the sample was homogenized, kept overnight at 4°C, and centrifuged at 1800 x g for 20 min. The supernatant was collected and the salt-soluble proteins (myofibrillar proteins, MP) were estimated. In the last extraction, the solid was mixed with 20 ml of 0.1 M NaOH, the sample was homogenized, kept overnight at 4°C, and centrifuged at 1800 x g for 20 min. The supernatant was collected and the alkali-soluble proteins (ASP) were estimated.

In the three protein fractions (SP, MP, and ASP) the extractable proteins were determined according to the method of Lowry and the protein profile was examined by SDS-PAGE.

Lowry Assay. Lowry's method was performed according to Deepachandi et al. (2020) with the following modifications: in a test tube were mixed well 1 ml of the extracted protein fraction with 5 ml of a reagent mixture A (2 % Na_2CO_3 in 0.1 N NaOH and 0.5 % $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1 % $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, in 50:1 ratio). The tube was incubated for 10 minutes at room temperature (23 °C). Folin and Ciocalteu's phenol reagent (1 N, 1 ml) was added, mixed well immediately, and incubated at room temperature for 30 minutes. A standard curve was made of bovine serum albumin and absorbance was read at 750 nm.

SDS-PAGE. The electrophoretic profiles were analyzed using polyacrylamide gel electrophoresis (PAGE) using a dissociating sodium dodecyl sulfate (SDS) buffer system in a discontinuous gel (6% stacking gel and 15% separating gel). SDS-PAGE was performed as described by Laemmli (1970) with an omniPAGE mini Cleaver electrophoresis (Model CVS10DSYS). Visualization of gels was realized with 0.2% Coomassie Brilliant Blue R-250 dye (SERVA Electrophoresis GmbH, Germany) for 20 min and discolored by immers-

ing in a solution containing 10% ethanol and 7% CH_3COOH overnight. Protein standards with molecular weights ranging from 10 to 250 kDa were used to determine the molecular weight of protein fractions. Data were analyzed by using TotalLab 1 D Analysis software (BioStep GmbH, Germany). To ease readability and data analyses, proteins were provisionally grouped into three categories: low molecular weight (LMW) proteins with molecular weights up to 50 kDa, medium molecular weight (MMW) proteins with molecular weights ranging from 51 to 150 kDa, and high molecular weight (HMW) proteins with molecular weights above 151 kDa.

Statistical analyzes. Presented data are the mean ± standard deviation of three independent experiments ($n = 3$). Data were analyzed by one-way analysis of variance (ANOVA) using Statgraphics Centurion statistical program (version XVI, 2009) (Stat Point Technologies, Ins., Warrenton, VA, USA). Mean differences were established by Fisher's least significant difference test for paired comparison with a significance level $p \leq 0.05$.

Results and Discussion

Protein content of soluble protein fractions

Table 3 shows the protein content of the three fractions (SP, MP, and ASP) isolated from the meat, as well as the total amount of protein in individual genotypes and weight groups. SP contains the glycolytic enzymes, creatine kinase, myoglobin, and parvalbumin (Ochiai & Ozawa, 2020). In our study, the sarcoplasmic fraction in the groups with lower live weight was shown significantly lower results than the groups with higher live weight. SP in Ab-1 and Ab-2 exhibited a protein content of 5.24 and 6.04% and represented 31.02 and 33.65% of the total protein content of *Acipenser baerii*. In *Acipenser gueldenstaedtii*, SP were 4.49 and 6.07% (25.63 and 30.86% of total protein content) but in their hybrids, the amount of SP in total protein was reduced to 25.14 and 29.24% for Hy-1 and Hy-2, respectively. The obtained results agreed with the data from the literature (Lopez-Enriquez et al., 2015), according to which SP represents 20–40% of the total fish muscle proteins.

According to Tahergorabi et al. (2011), MP makes up 66–77% of total proteins in fish muscle and are composed of myosin, actin, and regulatory proteins (such as tropomyosin, troponin, and actinin). The amounts of myofibrillar fractions in total protein profile were less in our study (42.12–60.86%), as opposed to SP fractions where the groups with lower live weight possessed a higher protein percentage than higher live weight groups. The highest yields of MP fractions

were observed at Ab1 and Ab2 (60.86 and 57.88%, respectively), followed by Ag-1 (55.48%) and the lowest – Hy-2 (7.75%). The highest yield of MP from the three fractions was due to the myosin, which is the main MP and represents approximately 60% of the total mass of skeletal muscle protein (Ochiai Y. & Ozawa H., 2020). According to Chen et al. (2016), extraction of fish muscle proteins with a 0.6 M KCl solution (pH 6.0) leads to greater solubilization of myosin due to his filament dissociation induced by the low ionic strength of the buffer solution. Probably, the highest yield of the MP fraction was a result of this peculiarity.

In addition to sarcoplasmic and myofibrillar proteins, the rest of the soluble proteins can be extracted in an alkaline medium. The solubility of proteins is usually reduced at the isoelectric point (around pH 5.5) while becoming gradually more soluble when the pH is lower or higher than in the isoelectric point. Tian et al. (2017) achieved maximum protein yield by 87.6% in alkaline conditions at pH 12.5 in their work with Common carp. Therefore, to extract the remaining amount of available soluble proteins, we used 0.1 N NaOH (pH 12-13) to receive the so-called ASP. From the results presented in Table 3, it is clear that their amount varied in a wide range – from 1.37 to 5.27%. Probably these variations in the results were a consequence of the conditions of the extraction processes. They might be affected by the sample/solvent ratio, the used centrifugation conditions, intrinsic (size and chemical composition), and extrinsic aspects (harvest season, temperature, and food availability) of the species (Lopez-Enriquez et al., 2015).

According to literature data (Marmon, 2012), in addition to the MP and SP, which have been discussed so far, there is a third group of proteins in fish – stroma proteins, such as collagen and elastin, that only constitute about 3% of the muscle proteins in total fish protein (Huss, 1995). The stroma proteins were not the subject of the present study due to the difficulties of the extraction process. Considering the total protein content in fish muscles (16-21%, Marmon, 2012), the data on the total amount of extracted proteins (Table 3) showed that satisfactory extraction

Table 3. Protein content in protein fractions of individual genotypes and weight groups

Group	SP, %	MP, %	ASP, %	Total protein content, %
Ab-1	5.24±0.24	10.28±0.31	1.37±0.04	16.89
Ab-2	6.04±0.20	10.39±0.29	1.52±0.06	17.95
Ag-1	4.49±0.09	9.72±0.29	3.31±0.08	17.52
Ag-2	6.07±0.35	10.05±0.30	3.55±0.26	19.67
Hy-1	4.86±0.27	9.53±0.28	4.94±0.22	19.33
Hy-2	5.38±0.19	7.75±0.39	5.27±0.22	18.40

of soluble proteins was achieved. Our results were similar to that obtained by Hashimoto et al. (1979) for the meat of sardines (*Sardinops melanosticta*) and mackerel (*Pneumatophorus japonicus japonicus*) where 33-37% of the muscle proteins were sarcoplasmic, 54-61% myofibrillar, and 4-5% alkali-soluble.

In general, it can be noted that in the study, in Russian sturgeon, Siberian sturgeon, and their hybrid, with increasing live weight of fish, SP and ASP fractions increased, while for the MP there was no well-defined trend.

Electrophoretic Profile (SDS-PAGE)

SDS-PAGE analysis revealed differences in the protein profiles of the SP, MP, and ASP fractions (Figure 1 A, B, and C).

Sarcoplasmic protein fractions

The electrophoretic profile of the SP is shown in Figures 1A, 2, 3, and 4. Protein bands with molecular weights (MW) ranging from 11 to above 250 kDa were detected, as low molecular weight (LMW) proteins with molecular weight up to 50 kDa among the three protein fractions were predominant. The distribution of protein fractions (%) is listed in Table 4.

The largest number of fractions (20) was registered at Russian sturgeon (Fig. 2), as the protein profiles of the two weight groups were similar. In Ag-2, the protein profile contained two additional bands – 121 and 187 kDa. The corresponding bands 14, 33, 37, 40, and 48 kDa from LMW, 62 and 100 kDa from MMW, were the most intense. The other 2 studied genotypes (Siberian sturgeon and Hybrid) showed a smaller number of fractions (19) and lacked fractions with a molecular weight higher than 250 kDa. Common to the three genotypes were fractions with molecular weights 11, 14, 16, 22, 25, 27, 33, 37, 40, 48, 55, 62, 70, 79, 100, 187, and 250 kDa (Figures 2, 3 and 4). In Ab1, Ab2 and Hy-1, a fraction from 12 kDa was observed.

It is known from the literature (Lopez-Enriquez et al., 2015) that the protein fractions with a molecular weight of 34, 40, 43, 50, and 94 kDa (attributed to glyceraldehyde phosphate dehydrogenase, aldolase, creatine kinase, enolase, and phosphorylase, respectively), present in a sarcoplasmic fraction from fish species, are related to aerobic metabolism of the fish cells. The myoglobin exists in a large amount in the sarcoplasm of aerobic muscle cells and has a molecular weight of around 17 kDa. Another sarcoplasmic protein is parvalbumin, with a molecular weight of about 11 kDa, which is a very stable protein, and does not aggregate. It remains soluble in the processing of fish (Kanamori et al., 2011) and is one of the major allergens in fish muscle (Ruthers et al., 2018).

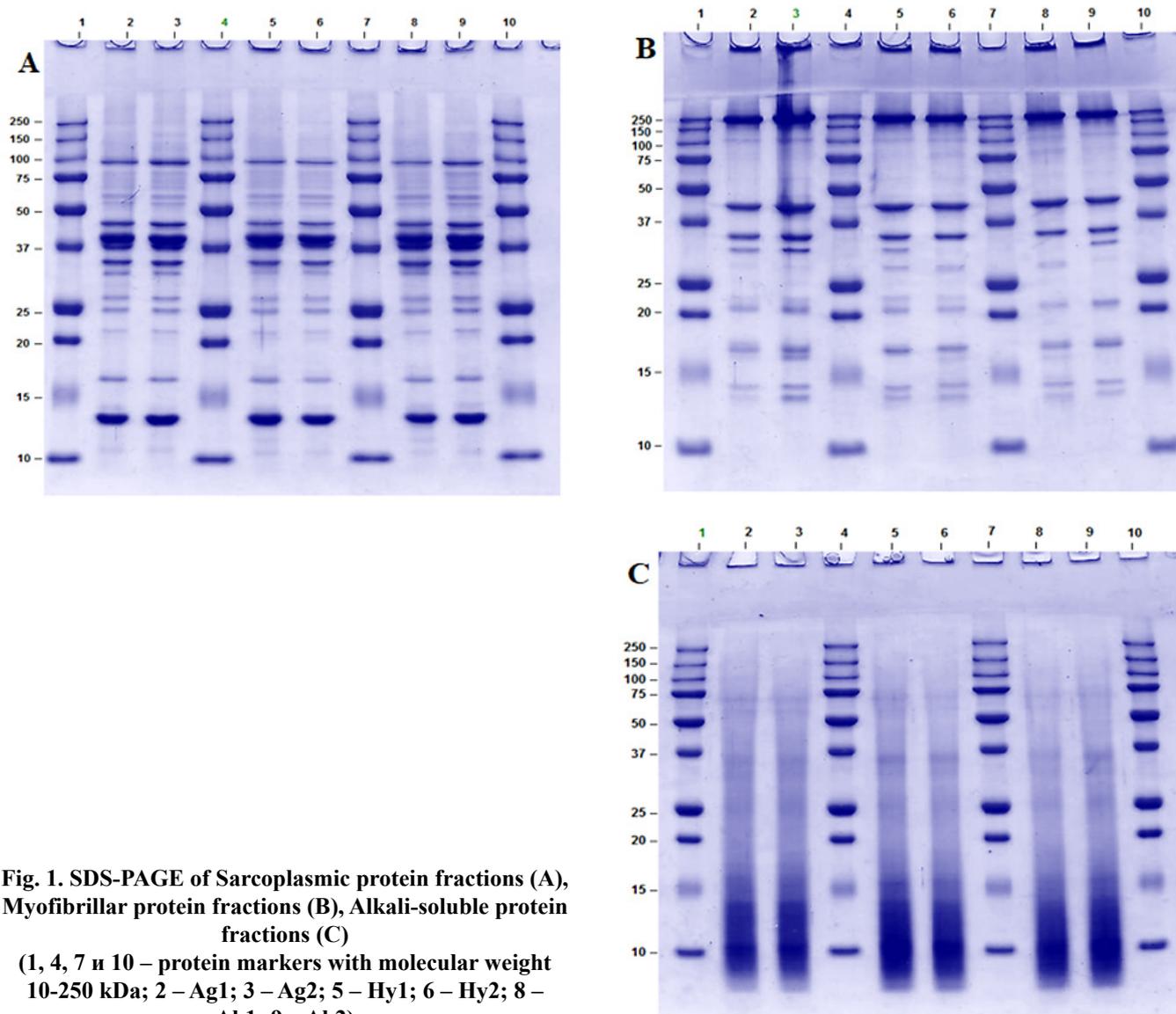


Fig. 1. SDS-PAGE of Sarcoplasmic protein fractions (A), Myofibrillar protein fractions (B), Alkali-soluble protein fractions (C)

(1, 4, 7 и 10 – protein markers with molecular weight 10-250 kDa; 2 – Ag1; 3 – Ag2; 5 – Hy1; 6 – Hy2; 8 – Ab1; 9 – Ab2)

Miofibrillaric protein fractions:

In contrast to the SP, in the composition of the MP in all studied genotypes and weight groups, a fraction with MW over 250 kDa was registered. Azadian et al. (2012) reported that the formation of protein bands with molecular weight over the 250 kDa can be explained by the dissociation of high molecular weight myosin and association of the protein to form a high molecular weight. Myosin is the main salt-soluble protein in skeletal muscle, constitutes approximately 60% (Ochiai & Ozawa, 2020) of the total mass of the protein. And due to its high total molecular weight (close to 500 kDa), it was registered in all samples. This hexamer consists

of two heavy chain subunits of approximately 200 kDa and four light chain subunits of approximately 20 kDa (Ochiai & Ozawa, 2020).

The presence of only 4 common fractions (MWs 13, 14, 17, and > 250 kDa) between the three genotypes makes a serious impression (Figures 5, 6, and 7). A lack of fraction with MW 250 kDa in the hybrids compared to the Russian and Siberian sturgeons is the most significant difference among studied fish. Its place in the two weight groups in the hybrids was occupied by a fraction with a slightly lower MW (231 kDa), which, however, occupied the highest proportion in the total protein profile (Fig. 6). Furthermore, fractions

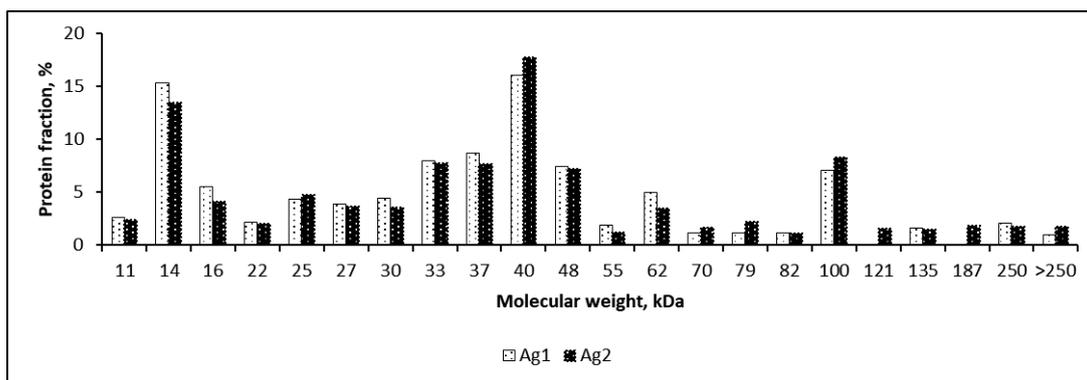


Fig. 2. Fractional profiles of SP from Ag-1 and Ag-2

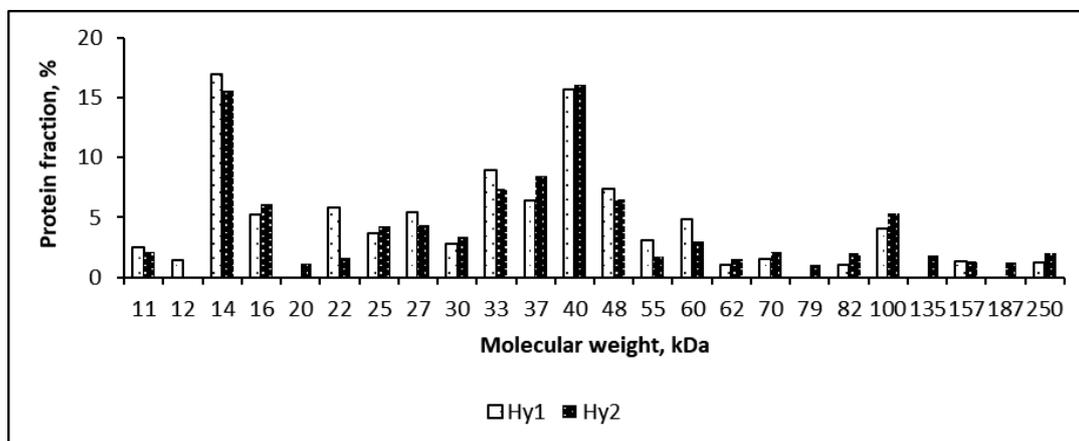


Fig. 3. Fractional profiles of SP from Hy-1 and Hy-2

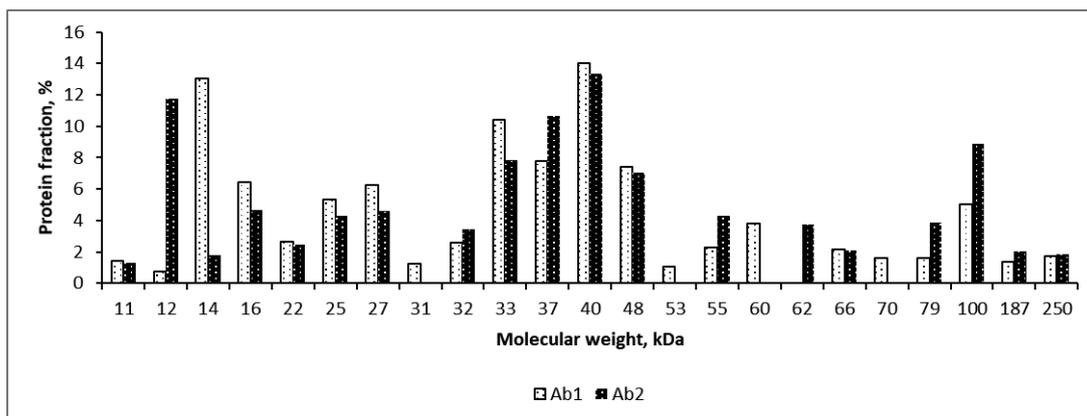


Fig. 4. Fractional profiles of SP from Ab-1 and Ab-2

that were not observed in the Russian and Siberian sturgeons were established in the hybrids (MWs 23, 29, 35, 49, 57, 84, and 103 kDa, respectively). According to the literature (Ochiai & Ozawa, 2020), fractions with molecular weights

of 19, 20, and 40 kDa belong to troponin, which is a complex of 3 subunits (T, I, and C). Lysozyme is a globulin with a MW of 14–17 kDa (Al-Holy & Rasco, 2006) and some of the bands in this area might be a consequence of its pres-

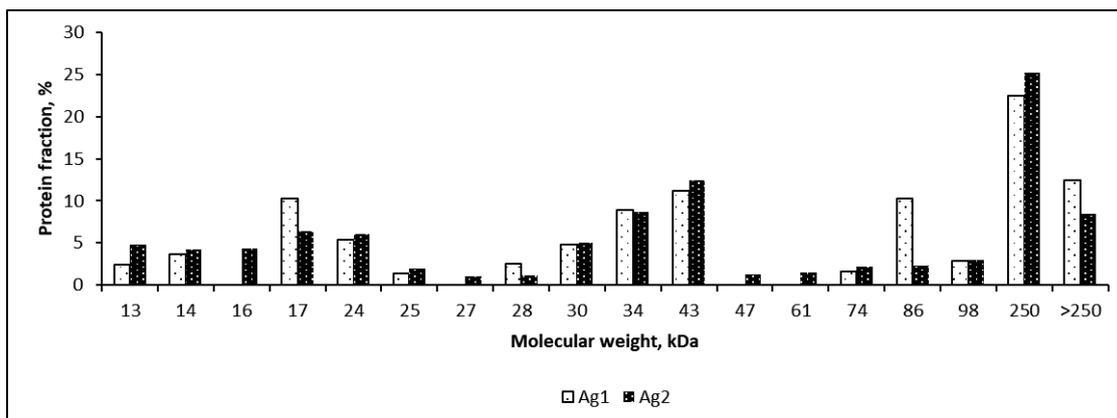


Fig. 5. Fractional profiles of MP from Ag-1 and Ag-2

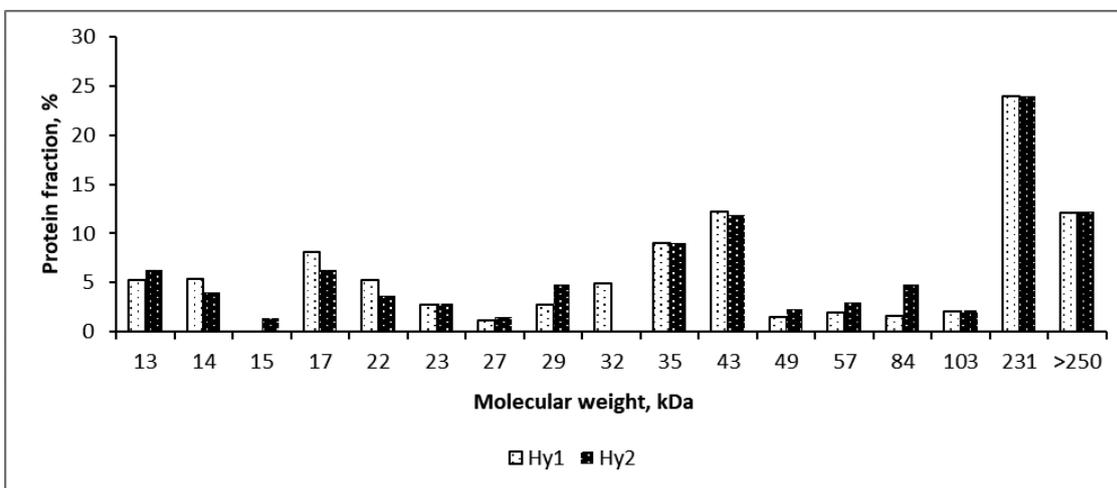


Fig. 6. Fractional profiles of MP from Hy-1 and Hy-2

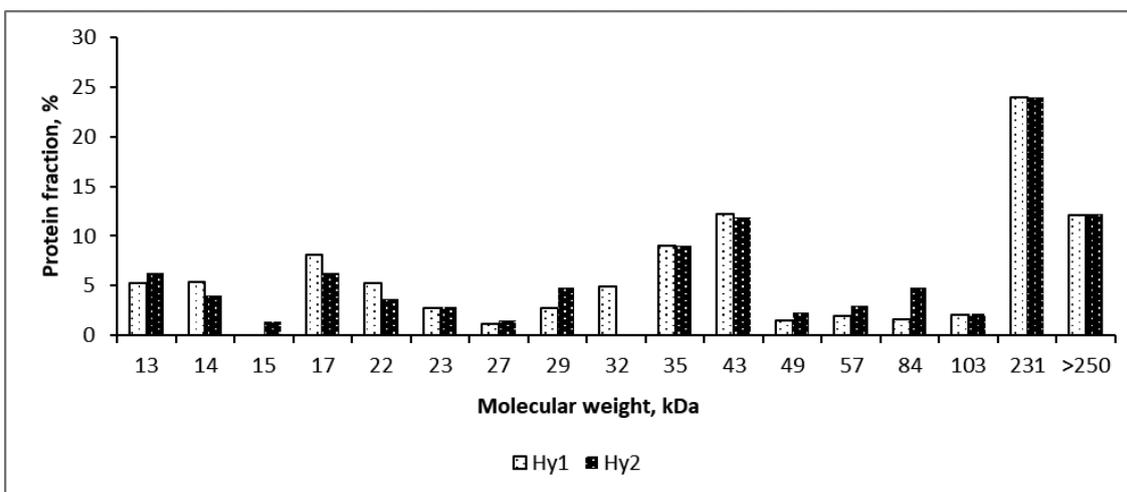


Fig. 7. Fractional profiles of MP from Ab-1 and Ab-2

ence. The same authors found a protein with a MW of around 97 kDa (a vitellin-like protein) in sturgeon *Acipenser transmontanus*. A similar band was also obtained for caviars from different sturgeon species by Chen et al. (1996).

Regarding the distribution of the individual molecular weight groups (Table 4), it is clear that in all three genotypes, the levels of LMW fractions (below 50 kDa) were the highest, as in the Russian and Siberian sturgeon profiles with an increase in the live weight their quantity increased (from 49.37% to 60.05% for Siberian sturgeon, and from 50.38% to 57.40% for Russian sturgeon). The trend was reversed for fractions with a molecular weight of 51-150 kDa (MMW) and HMW fractions (above 151 kDa). With increasing live weight, their share decreased (from 13.25% to 7.28% for Siberian sturgeon, and from 14.28% to 8.90% for Russian sturgeon). In the hybrids, the low molecular weight fractions were again with higher levels, but the trends described above for the two weight groups of Russian and Siberian sturgeon were not valid. In the hybrids, with increasing live weight of fish, decreased the quantity of LMW fractions (from 58.22% to 53.96%) and increased the number of fractions with a molecular weight of 51-150 kDa (from 5.67% to 9.91%). The relative distribution of HMW fractions in both weight groups of hybrid fish was the same (36.11% for Hy-1 and 36.13% for Hy-2).

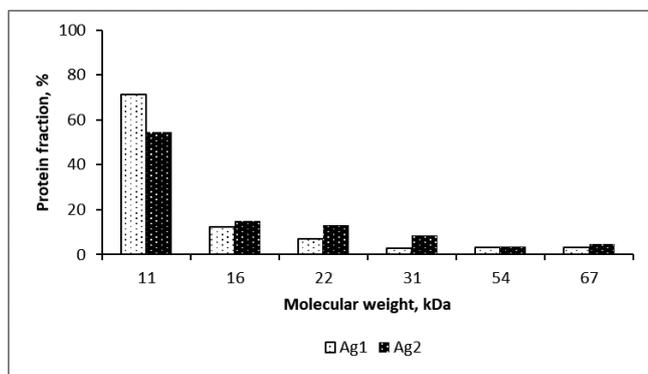


Fig. 8. Fractional profiles of ASP from Ag-1 and Ag-2

Alkali-soluble protein fractions:

The electrophoretograms of SDS-PAGE obtained for ASP from various genotypes and weight groups are shown in Figures 8, 9, and 10. HMW fractions were absent in all tested samples. On the other hand, low-molecular ones (11-37 kDa) were predominant (91-96%), while medium-molecular ones (54 and 67 kDa) accounted for an insignificant part of the protein profile (4-9%). The fraction with a molecular mass of 11 kDa formed the main part of the profile of the LMW fractions. These results suggest that some of the LMW fractions of sarcoplasmic and myofibrillar proteins remained

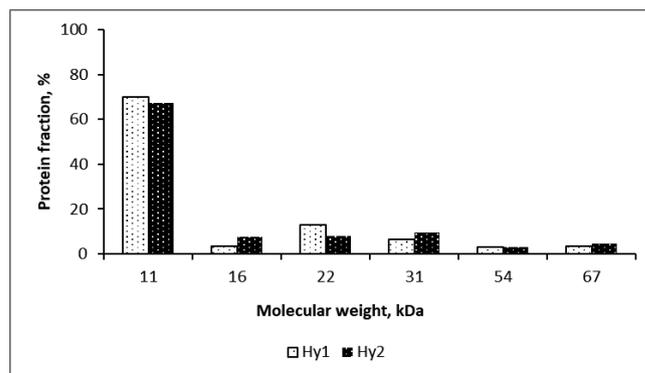


Fig. 9. Fractional profiles of ASP from Hy-1 and Hy-2

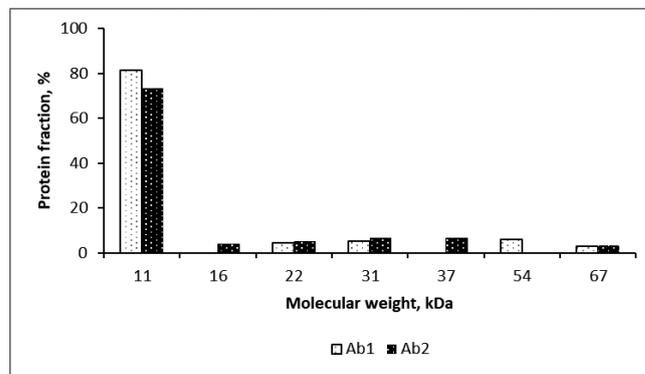


Fig. 10. Fractional profiles of ASP from Ab-1 and Ab-2

Table 4. Protein fraction distribution of weight groups from different genotypes

Group	Protein Distribution, %								
	LMW (< 50 kDa)			MMW (51-150 kDa)			HMW (> 151 kDa)		
	SP	MF	ASF	SP	MF	ASF	SP	MF	ASF
Ab-1	79.28	49.37	91.14	17.62	13.25	8.86	3.10	37.38	—
Ab-2	73.16	60.05	96.45	22.92	7.28	3.55	3.92	32.67	—
Ag-1	78.06	50.38	93.44	18.96	14.68	6.56	2.98	34.94	—
Ag-2	74.01	57.40	91.79	20.68	8.90	8.21	5.31	33.70	—
Hy-1	81.99	58.22	93.32	15.45	5.67	6.68	2.56	36.11	—
Hy-2	76.97	53.96	92.54	18.43	9.91	7.47	4.60	36.13	—

unextracted and in an alkaline environment their dissolution is favored. Furthermore, some of their high molecular weight fractions had hydrolyzed to peptides during the extraction process, which aggregated into low molecular weight proteins, resulting in the corresponding increase in the amount of LMW bands of ASP. According to Abdollahi & Undeland (2019), at pH 12, some actin and tropomyosin polypeptides are degraded, which confirms our assumptions as well. Due to the similarity in the electrophoretic profiles of the soluble proteins, the data cannot be used for species identification.

Conclusions

The electrophoretic patterns for extracts of different genotypes of sturgeon with distinctive bands, confirm that protein electrophoretic patterns can be used to differentiate fish species. SDS-PAGE is a rapid and reliable method for routine identification of genotypes based on the presence or absence of different bands in the SP and MP soluble fractions. Their visualisation allows to distinguish genotypes, which could help identify species and their hybrids.

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