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# NUTRITIONAL COMPOSITION OF SALMONIDAE AND ACIPENSERIDAE FISH EGGS

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#### Abstract

Analysis of the physicochemical properties of fresh eggs (raw material for caviar production) of the Salmonidae [sea trout (Salmo trutta L. 1758) and rainbow trout (Oncorhynchus mykiss, Walbaum 1792)] and the Acipenseridae [Siberian sturgeon (Acipenser baeri Brandt, 1869)], as well as sturgeon hybrids (Acipenser baeri Brandt, 1869 × Acipenser gueldenstaedti Brandt & Ratzeburg, 1833), included determination of basic physicochemical parameters (pH, dry weight, content of protein, fat, fiber and ash), amino acid composition and fatty acid profile. Compared to the Acipenseridae, Salmonidae eggs yielded a 22.5% higher total protein content, a 40.0% higher level of essential amino acids (EAA) and a 57.5% lower crude fat content. The sea trout eggs showed also a completely different fatty acids profile and hence values of lipid indices (lowest PUFA value – 11.72%, highest SFA value – 39.86%). The rainbow trout and sturgeon eggs had a similar fatty acid profile, and were characterized by a high nutritional and dietary value.

Key words: fish eggs, *Salmonidae* and *Acipenseridae*, physicochemical properties, amino acid composition, fatty acid profile

Fish and their products are an important component of human diet. Moreover, marine organisms are a valuable raw material in the nutraceutical and cosmetic industry (e.g. fish oil, collagen from the skin), fodder production (fish meals and oils); fish bones can be used for production of calcium dietary supplements, and fish eggs for caviar manufacturing (Bledsoe et al., 2003; Bubel et al., 2015; Ibrahim, 2015; Ninawe and Rathnakumar, 2008).

While the chemical composition and nutritional value of meat and fish products are quite well known (ElShehawy et al., 2016; Kaliniak et al., 2015; Mehta and Nayaka, 2017; Usydus et al., 2011), physicochemical properties of fish eggs are not fully understood yet, with respect to both their biological and physicochemical aspects. A single egg, i.e., the ovum, is a spherical structure. It is composed of the nucleus with the genetic information, the cytoplasm and a large amount of yolk containing fat, either in the form of droplets or dispersed within the cytoplasm. The whole egg is surrounded by the egg envelope (chorion). The physicochemical composition of fish eggs depends on the fish species and age and on environmental conditions in which the fish live (Bekhit et al., 2009; Niimi, 1983; Yanes-Roca et al., 2009). However, fish embryogenesis and ontogeny are still the focus of numerous studies (Brysiewicz et al., 2011; Davis et al., 2013; Korzelecka-Orkisz et al., 2010; Korzelecka-Orkisz et al., 2012).

Fish eggs for consumption, i.e., for the production of caviar, are obtained mainly from mature female sturgeons (*Acipenseridae*), less often from salmonids (*Salmonidae*) or other species from both marine and freshwater habitats as well as from farm breeding (aquaculture). Roe or hard roe is the fully ripe internal egg mass in the ovary, or the mass of eggs released by fish and certain marine animals, such as shrimp, scallops and sea urchins. As a seafood, roe is used both as a cooked ingredient in many dishes and as a raw ingredient. Eggs from a sturgeon, salmon or other fish are the raw base product from which caviar is made. Caviars are made from fish roe after the eggs have been graded, sorted, singled-out, salted or brined, and cured (Bekhit et al., 2009; Bledsoe et al., 2003; Ninawe and Rathnakumar, 2008).

According to the US data (USDA, 2016), there are fundamental differences between the eggs (after heat treatment) and caviar, which most probably result from technological processing. Compared to eggs, caviar is characterized by a much higher energy value, and contains twice as much fat, more than twelve times as much sodium, magnesium and iron, and also ten times more calcium. The vitamin A content is three times higher than in the eggs. Caviar is classified as a dainty food product with high nutritional and flavor value (Caprino et al., 2008; Wirth et al., 2000). Italy, Germany and France are the EU leaders of caviar production. USA, China, Iran and Russia are also large producers. In recent years, Poland has been gradually increasing its standing in the aquaculture of sturgeons, the main species from which the eggs are collected for black caviar production. According to various sources, the global production of caviar in recent years has amounted to 270–400 tons per year, and is projected to even double in the next few years (Bronzi and Rosnethal, 2014; Caviar Market Reports, 2018).

Polyunsaturated fatty acids, especially the omega-3 (EPA and DHA), which are not produced in the human body, play an important role in prevention of cardiovascular, neurodegenerative (Alzheimer's disease) and other diseases (Calder, 2018; Dutkowska and Rachoń, 2015; Siscovick et al., 2017).

Linoleic acid (C18: 2, *n*-6 LA) inhibits the formation and development of breast, skin, colorectum, and primary liver cancers (Chin et al., 1992; Stanley and Hunter, 2001). Conjugated linoleic acids have been found to have antimutagenic activity, to lower blood cholesterol (especially that of LDL), to counteract food-induced ath-

erosclerosis, to improve carbohydrate tolerance, and to reduce the body fat (Kritchevsky, 2000; Lawson et al., 2001).

Docosahexaenoic acid (C22:6, DHA) is an important component of the phospholipids of cell membranes and takes part in the regulation of their permeability. It occurs in the cell membranes of the whole organism, including the retinal cell membranes and the neurons of the cerebral cortex, where it constitutes half of all fatty acids. The DHA deficit in nerve cells is associated with disorders of the nervous system, increased sensitivity to stress, hyperactivity, aggression, and dyslexia and schizophrenia (Logan, 2004). In turn, eicosanoids formed from n-3 acids (e.g. eicosapentaenoic acid, EPA) have anti-inflammatory and anticoagulant properties, and suppress excessive vasoconstriction and cancer development (Noguchi et al., 1995; Tsaii et al., 1998; Rose and Connolly, 1991)

The aim of the study was to evaluate physicochemical properties of fresh eggs of sturgeons and salmonids, the basic raw material for caviar production, with a particular emphasis on their amino acid composition and fatty acid profile.

# Material and methods

#### **Biological material**

The materials consisted of fresh fish eggs collected from a total of 25 individual females caught in the middle of the spawning period. The eggs originated from salmonids (*Salmonidae*) [the sea trout (*Salmo trutta* L. 1758) and the rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792)] and sturgeons (*Acipenseridae*) [the Siberian sturgeon (*Acipenser baeri* Brandt, 1869) and a hybrid sturgeon of a female Siberian sturgeon (*Acipenser baeri* Brandt, 1869) and a male Russian sturgeon (*Acipenser gueldenstaedti* Brandt & Ratzeburg, 1833)].

The sea trout (TRT) was obtained in the Rega River (West Pomerania Region, North-West Poland). The eggs were collected from 7 females (55–74 cm body length; 1.6–4.2 kg weight). The rainbow trout eggs (RWT) were collected from 8 females from a breeding farm in Tarnów (Greater Poland Voivodeship). The fish length and weight ranged within 49–61 cm and 1.2–3.1 kg, respectively.

The sturgeon eggs were obtained from females kept at a breeding farm in Oleśnica (Greater Poland Voivodeship). The fish bred there originated from parents living in the Lena River, Russia. The Siberian sturgeon eggs (SSN) were collected from 5 females (135–141 cm body length, 28–36 kg weight). The sturgeon hybrids (SNH) were produced by interbreeding the Siberian sturgeon from the Lena (Russia) and the male Russian sturgeon from the Caspian Sea. The eggs were collected from 5 female hybrids (120–136 cm body length, 16.2–18.6 kg weight).

#### Eggs collection

The eggs were collected by massaging the abdominal walls of the females caught in mid-spawning, which guaranteed that the eggs were mature. Inactivated eggs (without contact with water and physiological fluids of females) were placed separately in sterile containers (150 ml) and transported to the laboratory at a constant temperature of  $1^{\circ}C\pm0.2^{\circ}C$  maintained in the containers.

# Laboratory analyses

Physicochemical analyses of the eggs were carried out according to standard laboratory methods (AOAC, 2012) with the authors' own modifications reflecting specific aspects of the analyses and the analytical equipment available. Part of the material was used to determine amounts of dry matter, crude ash and pH. The remaining part of the egg batch was lyophilized for analyses of protein, amino acids, fat and fatty acids as well as crude fibre content. The egg diameter was measured using a Nikon NIS-Elements computer image analysis as part of a set consisting of a Nikon TE-2000 S microscope, a Basler camera and a computer. Fifty eggs were measured each time.

#### Lyophilization technique

The eggs were lyophilized in the EDWARDS (UK) apparatus, with ISCEON MO 79 (DuPoint) as the refrigerant, and an RV 5 rotary pump use for pressing. The appropriate vacuum was applied at  $-60^{\circ}$ C, and the lyophilization proceeded until a solid mass of the material to be examined was obtained. The lyophilization coefficient was calculated.

## Dry weight determination

A portion of the material weighing approximately 2 g was transferred to a dried and weighed weighing bottle. The bottle was placed in a laboratory oven for 6 hours (at  $105^{\circ}$ C). Subsequently, the bottle was placed in a desiccator, cooled and re-weighed.

The dry weight was determined according to the following formula:

$$Dry weight = \frac{c-a}{b-a} \times 100 \ (\%)$$

where:

- *a* weight of empty, dried bottle (g)
- b weight of bottle with material before drying (g)
- c weight of bottle with material after drying (g)

# Crude ash determination

A 0.5–1 g sample (fresh egg mass) was transferred to a porcelain crucible and combusted in a muffle furnace for 8 hours at 600°C. After cooling, the crucibles were re-weighed and the ash content was calculated according to the following formula:

Crude ash = 
$$\frac{C-A}{B-A} \times 100$$
 (%)

where:

- A weight of empty crucible (g)
- B weight of crucible with material (g)
- C weight of crucible with material after combustion (g)

# pH measurement

The pH of the eggs (mixed and crushed mass) was measured with an INLAB (Germany) stationary pH meter. Each sample was measured 4 times. Prior to the measurement, the sample was dispersed in a small amount of distilled water.

#### **Crude protein determination**

The classic Kjeldahl method was used (AOAC, 2012). A weighed sample of lyophilized eggs was mineralized in a Kjeltec 2300 (FOSS CAUTION, Sweden) mineralizer, with 10 ml of concentrated  $H_2SO_4$  and a teaspoon of catalyst ( $K_2SO_4$  + CuSO<sub>4</sub>). The collector was 1% boric acid with a coloring indicator (a mixture of bromocresol green and methyl red). The titration was performed automatically (0.1 n HCL). The results are expressed as fresh weight %, with allowance made for the lyophilization coefficient.

## **Crude fat determination**

The analysis was conducted by extraction in a BUCHI B-811 (Switzerland) apparatus. Packets containing about 0.5 g of egg mass each in blotting paper were prepared, placed in a fat extraction vessel in a dryer, dried at  $100^{\circ}C \pm 0.2^{\circ}C$  for 30 minutes, and cooled in a desiccator. The packets were then placed in the BUCHI apparatus radiator and covered with petroleum ether. After extraction, the vessel with fat was returned to the dryer for 30 minutes, cooled in a desiccator, and re-weighed. The sample fat content was calculated according to the following formula:

Crude fat = 
$$\frac{c-b}{a} \times 100$$
 (%)

where:

- a sample weight (g)
- b weight of vessel before extraction (g)
- c weight of vessel after extraction (g)

# Determination of amino acid composition

The egg samples were hydrolyzed in 6 N hydrochloric acid for 24 hours at 110°C  $\pm 0.2$ °C. After hydrolysis, amino acids were separated by ion-exchange chromatography with post-column derivatization in reaction with ninhydrin in an AAA 400 amino acid analyzer (INGOS, Czech Republic). To determine their sulfuric amino acid content, samples of the material before hydrolysis were subjected to oxidation at 0°C for 16 hours in a 9:1 (vol:vol) mixture of formic acid and hydrogen peroxide. Tryptophan was determined spectrophotometrically after hydrolysis of the basic sample in 4M Lich and addition of 6 M HCl to obtain an acidic reaction. The samples prepared this way were centrifuged with a colorimeter reagent and 0.2% NaNO<sub>3</sub>. After 15 minutes, extinction was measured on a spectrophotometer at 590 nm in the presence of water. The results are expressed as g/kg fresh weight.

Based on these results we calculated essential amino acids (EAAs), nonessential amino acids (NAAs), EAA/NAA ratio and P-PER (predicted protein efficiency ratio). Free amino acids (ornithine, citrulline, homoserine) were not assayed. P-PER was calculated using one of the equations of Alsmeyer et al. (1974) as adapted by Adeyeye (2010): P-PER = -0.468 + 0.454 (Leu) - 0.105 (Tyr).

## Fatty acid profile determination

Determination of fatty acid profiles was conducted chromatographically. Approximately 0.5 g sample of lyophilized eggs was weighed, boiled (water bath) for 30 min with 2 ml 10% BF<sub>3</sub> in methanol at 72°C  $\pm$  0.2°C (the sample was heated under reflux condenser). Subsequently, 2 ml hexane and 2 ml water were added to the cooled sample, which was then vortexed for 2 minutes. After settling, the hexane layer was dried over anhydrous sodium sulfate and 1 µl aliquot was injected onto the capillary column of the gas chromatograph for qualitative and quantitative analysis.

The fatty acid profile was determined on a Clarus 580 GC (Perkin Elmer, USA) gas chromatograph equipped with a FID flame ionization detector and a ZB-WAX capillary column (i.d. 30 m × 0.25 mm, film thickness 0.25  $\mu$ m). The assays were carried out in He as a carrier gas (flow rate 20 m/s; temperature of the dosing chamber 250°C; detector temperature 450°C). The samples were analyzed using a preprogrammed temperature regime: 80°C for 1 min; temperature increase to 150°C at 45°C/min for 4 min; temperature increase to 230°C at 25°C/min for 7.5 min; temperature increase to 250°C at 25°C/min;, maximum temperature of 270°C maintained for 8.5 min. The whole analysis took 24.78 min. Chromatograms were qualitatively interpreted by comparing the retention times of fatty acid methyl esters of the sample examined with the retention times of ester standards of these acids (Supelco 37 Component FAME Mix).

The data obtained were grouped by acid type: saturated (SFA), unsaturated (UFA), monounsaturated (MUFA), polyunsaturated (PUFA), including omega-3 and omega-6. In addition, lipid quality indices (DFA, OFA, COX, AI, TI) were calculated using the appropriate formulas:

DFA - index of desirable fatty acids (C18:0+UFA),

OFA – sum of hypocholesterolemic fatty acids (Cl4:0 + C16:0),

COX – Cox value (Calculated Oxidizability Value) = [1 (18:1, %) + 10.3 (18:2, %) + 21.6 (18:3, %)] /100,

AI – atherogenic index =  $(C12:0 + 4 \times C14:0 + C16:0)/(PUFAn-3 + PUFAn-6 + MUFA)$ ,

TI - thrombogenic index = (C14:0 + C16:0 + C18:0)/(0,5MUFA + 0,5PUFAn-6 + 3PUFAn-3 + (PUFAn-3/PUFAn-6)).

#### Statistical treatment

The data were analyzed using the Kruskal-Wallis test (a nonparametric ANOVA equivalent), followed by the Mann-Whitney U test. For the basic properties (pH,

dry weight, protein, fat, fiber, ash), the significance of differences between all the groups, i.e., eggs from the four fish species, was assessed. For the amino acid composition, significance of differences between the fish groups was assessed for the sum of EAA and NAA, sum of AA as well as EAA/NAA and P-PER. For the fatty acid profile, significance of differences between the fish groups was assessed for SFA, UFA, MUFA, PUFA, sum of n-3, sum of n-6 and for lipid indices: UFA/SFA, MUFA/ PUFA, PUFA/SFA, DFA, OFA, n-3/n-6, COX and AI, TI. All the comparisons used the significance level of p=0.05. The data on the FA groups and lipid indices are given in Tables 3-4. In addition, the matrix of MUFA, PUFA, DFA, UFA, SFA, OFA, TL, Al and omega-3 and -6 in eggs of the four fish species was subjected to ordination to reveal possible gradients within the dataset, using the principal component and classification analysis (PCCA). A PCCA plot of egg samples and the projection of fatty acid concentrations measured in the eggs on the factor plane (Legendre and Legendre, 1998) gives information about similarities among samples and shows correlations between the original variable and the first two factors. These calculations were performed with the Statistica 13.1 (Statsoft 2017).

#### Results

The eggs of salmonids and sturgeons differed in size. The mean egg diameter of the sea trout and rainbow trout was  $5.28 \pm 0.44$  and  $4.46 \pm 0.34$  mm, respectively. The mean egg diameter of the Siberian and hybrid sturgeon was  $2.51 \pm 0.13$  and  $2.52 \pm 0.44$  mm, respectively (Figures 1–2).

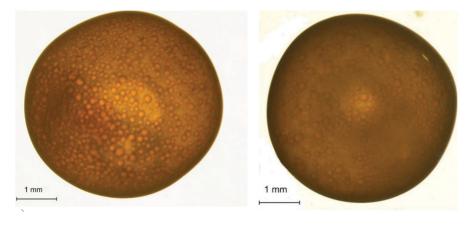


Figure 1. Salmonid eggs: trout (a) and rainbow trout (Oncorhynchus mykiss) (b)

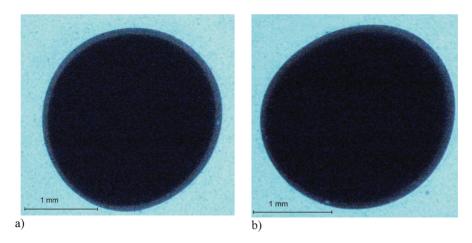


Figure 2. Acipenserid eggs: Siberian sturgeon (a) and sturgeon hybrid (*Acipenser baeri* × *Acipenser gueldenstaedti*) (b)

Data on the basic physicochemical egg properties of the fish species examined are shown in Table 1. In both cases, the egg pH was neutral (6.91-7.20), although the difference between TRT and SSN was significant (P<0.05). The egg dry weight was similar and ranged from 34.9 to 36.55%, only SNH and TRT differing significantly. The TRT eggs showed the lowest ash content (1.57%), other species yielding significantly higher levels. The TRT and RWT contained significantly higher amounts of crude protein compared to SSN and SNH. The maximum content was 24.93% (TRT). The highest fat content (10.46%) was found in eggs of the Siberian sturgeon eggs, the lowest level (4.26%) in eggs of the sea trout. The crude fat content in the sturgeon eggs was, on the average, more than twice that of the salmonids (P<0.05).

Variable	Trout	Rainbow trout	Siberian sturgeon	Sturgeon hybrid	
pН	7.20 b±0.16	7.14 b,c±0.19	6.91 a±0.15	6.98 a,c±0.12	
Dry weight (%)	36.55 a±1.15	36.05 a,b± 0.67	35.97 a,b±1.39	34.90 b±0.74	
Crude ash (%)	1.57 a±0.07	1.87 b±0.17	1.94 b,c±0.21	2.18 c±0.25	
Crude protein (%)	24.93 a±1.45	23.83 a±1.61	19.82 b±1.11	19.94 b±1.08	
Crude fat (%)	4.26 a±0.19	4.58 a±0.50	10.46 b±1.09	10.34 b±1.17	
Crude fiber (%)	0.19 a±0.08	0.31 c±0.09	0.59 b±0.22	0.40 c,d±0.09	

Table 1. Basic chemical properties of salmonid and acipenserid eggs

Values in the same rows with different letters (a,b,c,d) are significantly different (P<0.05).

As mentioned previously, fish eggs are a high nutritional value source of proteins and amino acids. Among essential amino acids (EAA), the most important contributor was leucine [23.27 g/kg fresh mass (TRT)], and the least important was tryptophan (Try) [1.64 g/kg fresh mass (SSN)]. A significantly higher EAA content was found in the salmonid eggs compared to the sturgeon eggs. Among the exogenous amino acids (NAA, including conditionally essential amino acids, EAAC), glutamic acid (Glu) occurred in the highest amount [maximum 33.24 g/kg of fresh mass (TRT)], while the cysteine (Cys) content was the lowest [3.08 g/kg fresh mass (SSN)]. A significantly higher level of NAAs was found in the salmonid eggs compared to the sturgeons. It is worth noting that the salmonid eggs contained significantly more crude protein, which could have affected the results (Table 2).

Amino acid	Trout Rainbow trout		Siberian sturgeon	Sturgeon hybrid					
	Essential amino acids (EAAs)								
Thr	$14.74 \pm 0.48$	12.18±0.41	10.12±0.60	9.91±0.27					
Val	17.93±.50	15.85±0.42	10.28±0.41	10.57±0.49					
Ile	$14.44 \pm 0.61$	12.36±0.34	9.35±0.45	9.62±0.38					
Leu	23.27±1.02	22.41±0.93	16.20±0.71	16.68±0.97					
Phe	12.51±0.65	12.53±0.56	7.61±0.36	7.73±0.42					
Lys	21.63±1.30	19.21±1.18	15.92±0.72	16.64±0.76					
Met	7.59±0.23	7.76±0.32	5.88±0.33	6.06±0.39					
Try	2.07±0.06	$1.84 \pm 0.11$	1.64±0.12	1.66±0.14					
Total EAA	114.00 a±3.16	104.13 c±1.77	76.99 b±4.32	78.85 b±2.76					
Non-essential amino acids (NAAs)									
Ala	21.01±0.75	19.86±0.78	12.46±0.64	13.20±0.56					
Tyr	$10.91 \pm .44$	9.84±0.49	7.39±0.40	7.19±0.37					
Pro	13.34±1.21	14.76±0.87	9.02±0.73	9.04±0.75					
Gly	6.65±0.19	5.93±0.18	5.63±0.18	5.78±0.15					
Asp	23.31±0.81	22.92±1.03	$18.32 \pm 0.80$	18.77±0.83					
His*	7.24±0.29	6.93±0.31	5.81±0.37	6.11±0.25					
Arg*	16.19±0.60	14.16±0.58	13.30±0.69	13.35±0.95					
Cys	3.97±0.10	$3.77 \pm 0.08$	3.08±0.12	3.37±0.08					
Ser	15.45±0.54	$14.18 \pm 0.41$	15.27±0.61	15.71±0.67					
Glu	33.24±0.81	30.80±0.78	31.26±0.98	32.35±1.05					
Total DAA	151.33 a±3.68	143.19 b±3.45	121.55 c±2.24	124.91 d±2.06					
ΣΑΑ	265.33 a±7.09	247.32 b±6.75	198.54 c±8.33	203.76 c±5.42					
EAA/DAA	0.75 a±0.02	0.73 a ±0.02	0.63 b±0.01	0.63 b±0.01					
P-PER	9.88 a±0.31	8.67 b±0.28	6.12 c±0.20	7.10 d±0.19					

Table 2. Amino acid composition (g/kg fresh weight;  $x \pm SD$ ) of salmonid and acipenserid eggs

\*Exogenous amino acids conditionally (EAAC), P-PER – predicted protein efficiency ratio, Glu – glutamic acid, Asp – aspartic acid.

Values in the same rows with different letters (a,b,c,d) are significantly different (P<0.05).

The fatty acid profiles of the fish eggs examined were quite diverse (Table 3, Figure 3). The largest differences in the fatty acid content concerned the sea trout compared to the other three species of fish. In particular, the TRT eggs contained much higher amounts of SFA, such as myristic acid (C14:0), stearic acid (C18:0), be-

henic acid (C22:0) and lignoceric acid (C24:0), and much lower amounts of fatty acids from the UFA group, i.e., eicosatrienoic acid (C20:3*n*-3), EPA (eicosapentaenoic acid) (C20:5*n*-3), erucic acid (C22:1*n*-9), DGLA (eicosatrienoic acid) (C20:3*n*-6) and DHA (docosahexaenoic acid) (C22:6*n*-3), than other species. Noteworthy is the low content of EPA + DHA in the TRT eggs (1.93%) compared to RWT (25.0%) as well as SSN (18.9%) and SNH (21.4% total FA).

Table 5. Faity acid contents (76, x±5D) in samonid and acipensend eggs								
Fatty acid	Trout	Rainbow	Siberian	Sturgeon				
I utty uotu	iiout	trout	sturgeon	hybrid				
Myristic acid (C14:0)	1.593±0.284	0.116±0.046	$0.316 \pm 0.067$	$0.506 \pm 0.040$				
Myristoleic acid (C14:1)	$0.132 \pm 0.011$	0.021±0.025	$0.034 \pm 0.014$	$0.022 \pm 0.022$				
Cis-10-pentadecanoic acid (C15:1)	$0.026 \pm 0.024$	$0.153 \pm 0.082$	$0.136 \pm 0.068$	$0.120 \pm 0.056$				
Palmitic acid (16:0)	14.61±0.411	15.86±1.582	$16.902 \pm 0.860$	$17.846 \pm 0.514$				
Palmitoleic acid (C16:1)	3.304±0.326	1.621±0.227	1.732±0.869	2,238±0.124				
Cis-10-heptadecenoic acid (C17:1)	$0.337 \pm 0.047$	$0.205 \pm 0.094$	$0.276 \pm 0.189$	$0.132 \pm 0.045$				
Stearic acid (C18:0)	$5.653 \pm 0.442$	1.715±0.279	$0.576 \pm 0.513$	$0.130 \pm 0.032$				
Oleic acid (C18:1) <sup>1*</sup>	17.01±1.243	29.099±2.446	$31.484{\pm}1.398$	31.740±1.006				
Linoleic acid (C18:2) <sup>2*</sup>	6.941±0.280	7.493±0.881	6.238±1.178	4,960±0.187				
Linolenic acid (C18:3) <sup>3*</sup>	2.160±0.156	5.295±1.470	3.791±0.821	$4.428 \pm 2.69$				
Arachidic acid (C20:0)	0.930±0.016	$0.075 \pm 0.037$	$0.104 \pm 0.027$	$0.086 \pm 0.041$				
Eicosenoic acid (C20:1)	$2.008 \pm 0.171$	1.241±0.560	$1.042 \pm 0.440$	$1.636 \pm 0.305$				
Eicosedienoic acid (C20:2)	1.066±0.111	0.536±0.516	$1.610\pm0.448$	1.308±0.222				
Eicosatrienoic acid (DGLA) (C20:3 <i>n</i> -6)	0.133±0.014	1.375±0.798	1.734±0.206	1.748±0.345				
Arachidonic acid (AA) (C20:4 <i>n</i> -6)	1.987±0.182	1.591±0.049	1.668±0.098	1.688±0.025				
Eicosatrienoic acid (C20:3 <i>n</i> -3)	1.837±0.137	5.328±0.868	3.956±2.059	2.356±0.700				
Eicosapentaenoic acid (EPA)	7.157±0.187	4.575±0.950	5.348±01.583	7.310±1.230				
(C20:5 <i>n</i> -3)	0.475±0.103	0.469±0.108	0.442±0.126	0.386±0.102				
Behenic acid (C22:0)								
Erucic acid (C22:1 <i>n</i> -9)	$2.385 \pm 0.085$	$0.689 \pm 0.204$	$0.968 \pm 0.380$	$1.066 \pm 0.416$				
Docosadienoic acid (C22:2)	$0.026 \pm 0.007$	0.700±0.274	$1.172 \pm 0.920$	$0.324 \pm 0.109$				
Lignoceric acid (C24:0)	$0.400 \pm 0.114$	$0.348 \pm 0.140$	$0.166 \pm 0.188$	$0.010 \pm 0.000$				
Docosahexaenoic acid DHA (C22:6 <i>n</i> -3)	26.910±1.089	18.853±2.177	18.682±1.398	18862±0.687				
Nervonic acid (C24:1)	0.461±0.118	0.389±0.023	0.336±0.112	$0.290 \pm 0.079$				
Unknown fatty acids	2.458±0.102	2.261±0.468	$1.240{\pm}0.492$	0,808±0.123				

Table 3. Fatty acid contents (%; x±SD) in salmonid and acipenserid eggs

1\* - two isomers, C18:1n-9c and C18:1n-9t, formed a single peak on the chromatogram.

2\* - two isomers, C18:2n-6c and C18:2n-6t, formed a single peak on the chromatogram.

3\* - two isomers, C18:3n-6 and C18:3n-3, were combined.

The fatty acid profiles expressed in percentages (Table 4) revealed differences between the salmonid and acipenserid eggs. The SFA content in the fish eggs examined ranged from 21.17% (SSN) to 23.93% (TRT), while the UFA contents ranged from 69.34% (SNH) to 77.38% (RWT). The proportion of MUFA was also the highest in RWT, while the PUFA and DFA levels were highest in TRT. The OFA proportion, too, differed significantly between the species, and was the highest in RWT. The

pattern described above was also reflected in the UFA/SFA, MUFA/PUFA, and PUFA/ SFA ratios, which were highest for SSN, SNH and TRT, respectively. The *n-3/n-6* ratio ranged from 1.32 (SSN) to 4.30 (TRT), which could reflect inter-specific differences. AI (atherogenicity index), an important index of fat quality, showed significantly lower values in eggs of RWT, SSN and SNH compared to TRT. The other index, TI (thrombogenicity index), was significantly lower in RWT compared to other species.

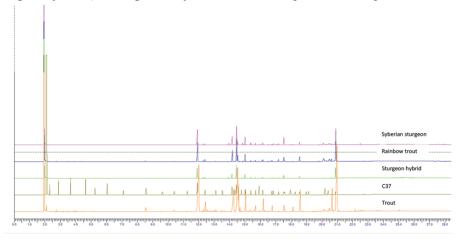


Figure 3. Chromatograms

Table 4 Fatt	y acid contents	(0/~.	v + SD	in	salmonid	and	acinenserid	eaac
Table 4. Fall	y actu contents	(70,	$x \equiv SD$	III	Sannonnu	and	acipenseriu	eggs

	2		1	66
Fatty acid	Trout	Rainbow trout	Siberian sturgeon	Sturgeon hybrid
			e	5
Total SFA	23.927 a±0.598	20.842 b±1.717	19.746 b±0.807	19.772 b±0.644
Total UFA	76.073 a±0.597	79.159 a±1.717	80.254 a±0.807	80.228 a±0.644
Total MUFA	25.008 a±1.208	33.414 a±2.401	36.056 a±1.022	37.244 a±1.060
Total PUFA	51.065 a±1.494	45.745 b±1.340	44.198 c±0.736	42.984 d±0.981
DFA	81.726 a±0.464	80.874 ab±1.556	80.830 b±0.676	80.358 b±0.624
OFA	16.197 a±0.521	15.974 a±1.582	17.218 a±0.976	18.352 a±0.518
UFA/SFA	3.182 a±0.102	3.830 a±0.390	4.073 a±0.211	4.063 a±0.168
MUFA/PUFA	0.491 a±0.037	0.732 a±0.067	0.816 a±0.033	0.867 a±0.042
PUFA/SFA	2.136 a±0.104	2.208 a±0.177	2.242 a±0.105	2.177 a±0.096
n-3	40.521 a±1.239	33.944 b±2.422	31.488 c±1.101	32.934 bc±0.619
n-6	9.753 a±0.464	10.565 a±1.482	9.928 a±1.578	8.418 b±0.532
n-3/n-6	4.162 a±0.201	3.317 b±0.771	3.266 b±0.603	3.926 ab±0.228
COX value	1.352 ab±0.046	2.206 a±0.325	1.776 b±0.214	1.785 b±0.063
AI	0.309 a±0.020	0.231 b±0.025	0.252 b±0.014	0.269 b±0.010
TI	0.144 a±0.006	0.132 b±0.010	0.140 ab±0.004	0.142 ab±0.006

Values in the same row with different letters (a,b,c,d) are significantly different (P<0.05).

SFA. saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, including *n*-3 and *n*-6; DFA, index of desirable fatty acids (C18:0+UFA); OFA, sum of hypocholesterolemic fatty acids (C14:0 + C16:0); COX, Cox value (Calculated Oxidizability Value) = [1 (18:1%) + 10.3 (18:2%) + 21.6(18:3%)]/100; AI, atherogenic index = (C12:0 + 4 × C14:0 + C16:0)/(PUFA*n*-3 + PUFA*n*-6 + MUFA); TI, thrombogenic index = (C14:0 + C16:0 + C18:0)/(0.5MUFA + 0.5PUFA*n*-6 + 3PU-FA*n*-3 + (PUFA*n*-3/PUFA*n*-6)).

Correlations between FA groups and lipid indices (LI) in the fat of the fish eggs examined are shown in the PCCA plot (Figure 4). PUFA, Al, Tl, SFA and n-3 indices were correlated, as were MUFA and UFA and n-6 and DFA. SFA were negatively correlated with UFA as well as MUFA and n-3 as well as OFA and DFA. It is also worth noting that fatty acid profiles of the trout eggs differed distinctly from the profiles established for other species.

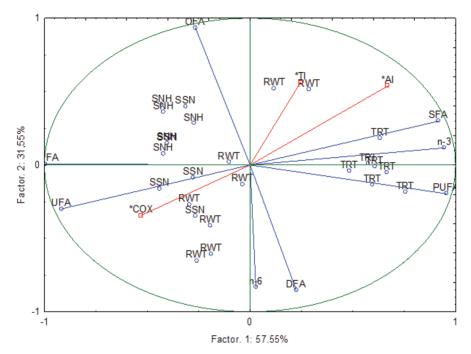


Figure 4. PCCA ordination of eggs from the 25 individuals examined, based on fatty acid contents in different fish species (TRT, trout; RWT, rainbow trout; SSN, Siberian sturgeon; SNH, sturgeon hybrid)

#### Discussion

According to American data, fish eggs (after thermal treatment) contain, on the average, 29% protein and 8% fat (Caviar Market Reports, 2018). In this study, values were similar for eggs of the sea trout and rainbow trout, with considerably lower values being obtained for eggs of the sturgeon and its hybrid. The dry weight of carp eggs contributed by crude protein, crude fat and crude ash was 34.7%, 73.44, 8.38 and 3.78%, respectively (Oroian et al., 2013). Our results proved to be similar with respect to dry weight and crude ash, the crude protein level in our study being lower. In eggs of the Nile tilapia, the moisture and crude protein contents ranged from 56.13 to 59.09 and 58.65 to 65.30%, respectively, twice as high as the levels found in our study. Similarly, the crude fat level in the Nile tilapia was 32.80–35.67%. i.e., over

3 times higher than in the sturgeon eggs, and 6 times higher than in the eggs of the sea trout and rainbow trout. Ash accounted for 2.18–4.55% of the dry weight, again higher than the level found in the present study. The chemical composition of eggs of the spawning broodstock are to some extent affected by the protein and energy content in feed (diets) as well as by many other factors, including physiological variables (Abdel-Fattah and Mamdouh, 2008). For example, pH, osmolality and moisture content were lower in mature salmon roe, but the egg size, viscosity, protein, crude lipid and ash contents were all higher compared with those in eggs of the immature chinook salmon (Bekhit et al., 2009) In terms of basic physicochemical characteristics, the composition of eggs differs from that of fish meat (ElShehawy et al., 2016; Ibrahim, 2015; Niimi, 1983) or chicken and quail eggs (Nutrient Composition Tables, 2017; Oroian et al., 2013; Vakili and Majidzadeh Heravi, 2016). However, there are quite considerable between-species differences, particularly with respect to the crude protein content.

Gunasekera et al. (1996) found the highest contents of histidine (His) among EAAs, and proline (Pro) among NAAs in the Nile tilapia eggs, while the  $\Sigma AA$  content increased with the dietary protein level. A similar relationship between His and crude protein content was evident in our study. Similarly, eggs of the golden shiners (Notemigonus crysoleucas) showed the highest amounts of glutamic acid and leucine (Leu) and the lowest contents of tryptophan (Try) (Lochmann et al., 2007). The EAA/NAA and P-PER ratios in salmonids were significantly higher than those in sturgeons. Interestingly, the highest P-PER (9.98) was found in the sea trout eggs, and it was almost 4 times higher than that in, for example, the meat of the Nile bolti (2.53) (Ibrahim, 2015). Certainly, many other quality parameters of amino acids (Adeyeye, 2010) should be considered from the point of view of human nutritional needs (Reeds, 2000). The amino acid composition of fish eggs differs from that of fish meat (Mehta and Nayaka, 2017; Usydus et al., 2009) and of mammalian milk (Rafig et al., 2016) or poultry eggs (Nutrient Composition Tables, 2017). The main criterion for the assessment of protein value is the content of essential amino acids, including sulfuric amino acids, which are higher in TRT and RWT than in SSN and SNH.

Among many other fish species, the highest level of EPA + DHA (44.4%) was found in the European hake (*Merluccius merluccius*) eggs, and the lowest (8.9% total FA) in eggs of the catla (*Catla catla*) (Kaliniak et al., 2015). On the other hand, Bekhit et al. (2009) reported a high proportion of EPA and DHA in the fat of salmon eggs, the fatty acid profile being independent of the fish age. The profile of polyunsaturated fatty acids (PUFA) changed over the spawning season (from 37.5% to 29.4% of total FA) and the egg quality was at its best in May, June and July. Eggs of the common snook (*Centropomus undecimalis*), which yielded a higher concentration (13% total FA) of docosahexaenoic acid (DHA), had higher fertilization, hatching and larval survival rates (Yanes-Roca et al., 2009). Amounts of C16:0 (25.45–28.07%) and C18:1 (17.25–19.19%) acids in eggs of the golden shiners (*Notemigonus crysoleucas*) depended on the egg collection period (March – June) (Lochmann et al., 2007). In our study, the most abundant were C18:1 (17.01–31.74%), followed by C16:0 (14.61–17.846%) acids. There are some data indicating differences in fat content between the sturgeon, carp and tuna (from 5.6 to 24.4%) as well as in SFA (13–52.8%), MUFA (20.3–49.15%) and PUFA (11.09%–49.9%) between freshwater and marine fish. The lowest *n*-3/*n*-6 ratios were typical of the egg fat of the Atlantic cod (1.79) and carp (1.9), the highest ratios (up to 22.7) being found in eggs of the Caspian kutum (*Ruitilus fristi katum*) (Kaliniak et al., 2015). Other lipid indices, such as OFA, AI and TI also varied in fat of the eggs of the fish species examined.

Attia et al. (2015) reported the AI and TI values for table eggs to range from 0.458 to 0.533 and 0.395 to 0.784, respectively, depending on the source (farm). In our study, these values were definitely different: 0.231–0.309 and 0.132–0.144 for AI and TI, respectively. The indices were completely different in broiler and fattener meat and cow milk, as the fat SFA and UFA contents are different (Attia et al., 2017; Bodkowski et al., 2016; Hanczakowska et al., 2015; Pilarczyk and Wójcik, 2015). Ulbricht and Southgate (1991) consider AI and TI to be better indices of atherogenicity and thrombogenicity than the PUFA/SFA ratio; in general, the lower AI and TI, the more beneficial for human health the fat is. This is because not all SFA are hypercholesterolemic, and, in addition to polyunsaturated acids (PUFA), the protective activity is also attributed to monounsaturated acids (MUFA).

In conclusion, it should be noted that the eggs of the fish species examined are rich in PUFA, including EPA and DHA, their contents approximating or exceeding those in the meat or liver fat of many marine fish (ElShehawy et al., 2016; Kaliniak et al., 2015; Mehta and Nayaka, 2017; Usydus et al., 2011), which is very advantageous from the standpoint of dietary recommendations and medical prophylaxis (Simopoulos, 2016; Swanson et al., 2012). Our study demonstrated that the fatty acid content in fish eggs showed considerable variability and that the fatty acid profile was species specific. Comparison with the literature data shows the need of further research in which not only the fish species, but also the time of egg collection should be taken into account.

# Conclusion

In terms of basic physicochemical parameters, salmonid eggs are richer in total protein and contain less crude fat than sturgeon eggs. The salmonid eggs are also richer in essential amino acids (EAA) than the sturgeon eggs. On the other hand, the trout eggs showed the highest PUFA level, the lowest SFA level being typical of the sturgeon eggs. Both sturgeon and rainbow trout eggs prove a very valuable raw material for caviar production.

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