

Fatty acids and fat soluble vitamins content of Black Sea round goby (*Neogobius melanostomus*, Pallas, 1814) during fishing seasons

Merdzhanova A.^{1*}; Dobрева A.D.¹

Received: August 2017

Accepted: September 2018

Abstract

The aim of the present study was to determine and compare the seasonal changes in the proximate composition, fatty acids profile and fat soluble vitamins content in spring and autumn goby (*Neogobius melanostomus*) caught from the Bulgarian Black Sea waters. The proximate composition (moisture, protein and total lipid) has been determined according to AOAC (1990). Analysis of fatty acid methyl esters has been performed by GC/MS system. Vitamins A, D₃ and E were analysed using RP-HPLC system. Protein was in the range 18.10 – 18.75 %, fat content was: 1.60-2.61g 100g⁻¹ wet weight (w.w.). The fatty acids (FA) and vitamins contents showed significant seasonal changes. The spring goby was characterized with low saturated fatty acids (SFA, 31.8 %), high mono unsaturated fatty acids (MUFA, 34.86 %) and polyunsaturated fatty acids (PUFA, 33.34 %). In both seasons omega-3 (n-3) PUFA levels were higher than omega-6 (n-6) PUFAs. Higher amounts of alpha-tocopherol (614.9 µg 100g⁻¹w.w.) and all-trans retinol (14.8 µg 100g⁻¹ w.w.) were found in autumn samples, whereas cholecalciferol (4.9 µg 100g⁻¹ w.w.) - in spring goby. Regardless of the observed seasonal changes of nutritional quality, goby species is an excellent source of analysed components, especially of n-3 PUFA, and can be recommended for healthy human diet.

Keywords: Black Sea, *Neogobius melanostomus*, Omega-3 fatty acids, Fat soluble vitamins

1-Department of Chemistry, Faculty of Pharmacy, Medical University, Varna, 9000, Bulgaria

*Corresponding author's Email: a.merdzhanova@gmail.com

Introduction

Many studies have been conducted on the effects of fish consumptions in the prevention of coronary heart disease, atherosclerosis, thrombosis and others. In the past few years, the increase in the supplementary intake of vitamin D₃ and omega-3 polyunsaturated fatty acid (n-3 PUFA) consumptions by the general population was recommended (Jump *et al.*, 2012; Stark *et al.*, 2016). This could result in a concomitant rise in brain serotonin levels and function, therefore increasing normal cognitive function. In comparison to the other European countries (23 kg year⁻¹ per capita) the fish consumption (n-3 PUFA and vitamin D₃ respectively) in Bulgaria is traditionally low (5.1 kg year⁻¹ per capita). Bearing in mind all these facts, it would be beneficial to increase fish consumption especially native Black Sea fish like round goby. It is well known that marine fish are richer sources of nutrients mentioned above compare to freshwater fish (Tocher, 2003; Ghomi *et al.*, 2014). Some species inhabiting low salinity or brackish waters may also contain the high amounts of n-3 PUFA. One interesting but invasive Black Sea species, which inhabit marine and brackish waters, is round goby. After invasion in Black Sea region this small, resident (non-migrated) Gobies species acquire even economic significance. According to official statistics of the fishing on Gobies in Bulgaria, their catches have kept the second place during the last two decades and the target Gobiid species is *Neogobius melanostomus* (Vassilev *et al.*, 2011; National Strategic Plan for Fisheries and Aquaculture 2007-2013). This demersal fish is of commercial interest for the Black

and Azov Sea neighbouring countries. Invasive round goby has important effects on the function of native food chain in the aquatic ecosystems and also represent food source for other benthic predators (sharks, rays, sturgeons, Percids). On the other hand, the evaluation of nutritional quality of this resident and widespread species will be useful for consumers. Determination of the proximate composition (proteins, carbohydrates, lipids and moisture contents) is the first step, which is used to ensure that fish tissues have good nutrition quality and that it meets the requirements of food regulations and commercial specifications (FAO, 2010). In the recent years, a number of investigations have assessed the proximate composition of fish species as shad, horse mackerel, garfish and goby from Southeast (Guner *et al.*, 1998; Boran and Karaçam, 2011) and Northwest (Creteanu *et al.*, 2008) part of the Black Sea. The chemical composition and fatty acids content of fish is known to be affected by different abiotic factors including seasons. However, studies of seasonal changes in the proximate and fat soluble components (fatty acids, vitamins) of the commercially important round goby (*N. melanostomus*) from Bulgarian part of the Black Sea are lacking. Furthermore, the fish demand has been increased in the recent years and consumers' interests turn into the investigated species (Health of the Nation Report, 2009). Unfortunately, the available information about the nutrition quality of round goby edible tissues is still insufficient and incomplete. Therefore, the aims of this work are to assess the seasonal changes of the proximate composition, fatty acids profile and fat soluble vitamins

content of edible tissues of the round goby (*N. melanostomus*) caught from the Bulgarian Black Sea coast.

Materials and methods

Sample preparation

All fresh fish specimens were purchased from Varna local fish markets during fishing season in spring (March 2015) and autumn (September 2015). The fish species were caught from Varna Region of the Bulgarian Black Sea coast. Specimens of similar body weight and length have been selected from captured species in both seasons. Biological characteristics, such as body weight (g), length (cm) and habitats are noted in Table 1. A minimum of twenty-five specimens have been randomly selected from 2 kg batches during each individual season. Fish were immediately gutted, filleted, minced and then stored at -20°C prior to analysis.

Table 1: Biological characteristics of analysed goby samples (mean \pm SD).

Season	Sampling location	Length (cm)	Weight (g)
Spring	Varna	16.0 \pm 1.1	65.0.0 \pm 1.0
Autumn	Varna	17.5 \pm 1.5	74.0 \pm 2.5

SD - standard deviation

Hydrographic parameters

During the study period, temperature ranged from 5.9°C in March 2015 to 24.4°C in September 2015 (www.stringmeteo.com/synop/seawater.php, BG). Salinity varied from 17.5 ‰ (March 2015) to 16.5‰ (September 2015).

Proximate composition analysis

The homogenized fish tissues (2.000 ± 0.005 g) were dried at $105\pm 2^{\circ}\text{C}$ in an air oven to constant weight for 16-18 hours (AOAC 950.46). The moisture has

calculated as weight loss. Crude protein content has determined by Kjeldahl method (BDS 9374:1982). Total lipids (TL) were determined according to Bligh and Dyer procedure (1959) and results were presented as g per 100 g wet weight ($\text{g } 100\text{g}^{-1}\text{w.w.}$). Carbohydrate content has calculated by subtracting the total of protein, TL and water as a whole. Energy value has calculated by multiplying fat and protein with appropriate coefficients (4.0 kcal g^{-1} for proteins and carbohydrates, 9.0 kcal g^{-1} for lipids) (FAO, 2010).

Fatty acids analysis

Fatty acids compositions of TL in edible fish tissues have determined by Gas chromatography (GC) of the corresponding methyl esters. The residual lipid fraction was methylated by base-catalysed transmethylation according to BDS EN ISO 12966-2:2017. Gas chromatography has performed by a FOCUS Gas Chromatograph with Polaris Q MS detector (Thermo Scientific, USA). Three replicate GC analyses have performed. The values of FA have expressed as percentage of total FAs mass as a mean value \pm standard deviation (SD) according to BDS EN ISO 12966-4:2015. The percentage values of long-chain n-3 FA (EPA+DHA) were recalculated to $\text{g } 100\text{g}^{-1}$ of goby edible tissues according to FAO/INFOODS Guidelines (2012). All of the chemicals used in the experiments were at analytical grade and GC grade (Sharlau, Sharlau Sourcing Group, Spain).

Nutrition quality indices (NQI)

Nutrition qualities have been estimated by several indices and ratios based on FAs composition: the indices of atherogenicity

(AI), thrombogenicity (TI), cholesterolemic index (h/H); n-6/n-3 and PUFA/SFA ratios, according to Simopoulos (2013). Ulbritt and Southgate (1991) suggest two indices – AI and TI which might better describe the atherogenic and thrombogenic potential of unsaturated FA. h/H presents the functional effects of different PUFAs of cholesterol metabolism (hypo- and hypercholesterolemic effect), and calculated according to Santos-Silva *et al.* (2002).

Extraction of fat soluble vitamins and HPLC analysis

The extraction and quantity analysis has performed using the method of Dobreva *et al.* (2011). Three fat soluble vitamins (A, E and D₃) have been analysed simultaneously using HPLC system (Thermo Scientific Spectra SYSTEM) equipped with analytical column ODS2 Hypersil™ 250×4.6mm, 5µm. The quantitation has done by the method of external calibration comparing the chromatographic peak areas of the

corresponding standards (Retinol solution, Fluka; DL-alpha Tocopherol, Supelco; Cholecalciferol, Supelco). The results are expressed as µg 100g⁻¹ wet weight (µg 100 g⁻¹ w.w.).

Statistical analysis

The results of analysis are presented as mean values±standard deviation (SD). Column statistics has used for calculation of the means and standard deviations. Student's t-test has used to evaluate the differences between the means. Statistical significance has indicated at $p<0.05$. Statistical analysis has done using Graph Pad Prizm 5, USA software.

Results

Proximate composition

The seasonal changes in the proximate composition of the round goby have been shown in Table 2. In this study, it has been found an inverse relationship between the water, protein and lipid contents of goby fish tissues.

Table 2: Seasonal changes in proximate composition and energy values of the goby (means±SD).

Components	Moisture %	Lipid g 100g ⁻¹ w.w	Protein g 100g ⁻¹ w.w	Energy kJ 100g ⁻¹ w.w
Spring	77.90 ± 1.05	1.60 ± 0.07	18.73 ± 0.50	373.0±0.20
Autumn	75.40 ± 0.95***	2.65 ± 0.15***	18.10 ± 0.45**	389.1±0.35**

SD - standard deviation; Statistical significance of differences between seasons (*** $p<0.001$; ** $p<0.01$)

The moisture content in spring goby was higher ($p<0.001$) than in autumn samples (Table 2). Total lipid (TL) content increased up to 2.65 g 100 g⁻¹ w.w., whereas protein levels more insignificant decreased in autumn season ($p<0.001$). According to FAO (2010), protein content of fish is considered low if, it is below 15 %. Moreover, in accordance with supplement of Regulation (EC) No

1924/2006 and Commission Regulation (EC) No 116/2010 the analysed round goby in both seasons have showed protein contents in range of 18.10-18.73 g 100g⁻¹ w.w. and can be classified as high in protein. The lipid level in particular has high significance for the calorie content of fish, with implications for calculations in dietary studies and databases. In this study, energy values ranged from 373.0 kJ 100g⁻¹

w.w. (spring) to 389.1 kJ 100g⁻¹ w.w. for autumn goby samples ($p<0.01$). There is no data available in literature for seasonal changes in the proximate composition and energy value of the round goby caught from the Bulgarian Black Sea coast.

Fatty acids composition

The seasonal changes in FAs composition of the round goby edible tissues are given in Table 3.

Table 3: Seasonal variation of fatty acids profile, groups (FA, % of total FA), ratios, nutritional quality indices and EPA+DHA contents (g. 100g⁻¹ w. w.), of goby edible tissues (mean±SD).

Fatty Acid	Spring	Autumn	Fatty Acid	Spring	Autumn
C 12:0	1.02 ± 0.12	2.35 ± 0.20***	C 18:3 n-3	0.54 ± 0.40	4.10 ± 0.80***
C 14:0	2.50 ± 0.25	3.42 ± 0.60***	C 20:5 n-3	1.89 ± 0.15	3.53 ± 0.9***
C 16:0	24.00 ± 1.38	17.90 ± 0.87***	C 20:4 n-6	3.12 ± 0.30	4.67 ± 0.65**
C 17:0	0.05 ± 0.01	0.77 ± 0.60	C 20:2 n-6	nd	1.70 ± 0.20
C 18:0	3.08 ± 0.20	5.82 ± 0.56***	C 20:3 n-3	0.08 ± 0.01	0.74 ± 0.01
C 20:0	1.05 ± 0.02	2.92 ± 0.45	C 20:3 n-6	0.06 ± 0.01	2.50 ± 0.15***
C 21:0	nd	nd	C 22:6 n-3	16.30 ± 1.02	12.50 ± 0.30***
C 22:0	0.05 ± 0.01	1.80 ± 0.22	C 22:2 n-9	0.06 ± 0.02	0.50 ± 0.09
C 23:0	nd	0.13 ± 0.01	PUFA	33.38	35.36 ***
C 24:0	0.05 ± 0.01	1.00 ± 0.05	Σ n-3	18.81± 0.80	20.87±1.10***
SFA	31.75	36.11 ***	Σ n-6	14.50 ± 0.30	14.00 ± 0.65*
C 14:1n-5	0.62 ± 0.01	1.50 ± 0.21	n-6/n-3	0.77 ± 0.02	0.67 ± 0.03
C 16:1n-7	16.80 ± 0.89	7.80 ± 0.30***	PUFA/SFA	1.05 ± 0.10	0.98 ± 0.05
C 17:1	0.20 ± 0.36	0.77 ± 0.12	DHA/ EPA	8.62± 0.35	3.54± 0.24***
C 18:1n-9	11.94 ± 0.60	10.00 ± 0.61*	C 16:0/C 18:1n-9	2.00 ± 0.15	1.79 ± 0.10*
C 20:1n-9	1.90 ± 0.11	3.26 ± 0.28**	AI	0.50 ± 0.02	0.36 ± 0.01
C 22:1n-9	2.00 ± 0.15	2.65 ± 0.25*	TI	0.59 ± 0.03	0.32 ± 0.01
C 24:1n-9	1.40 ± 0.05	2.55 ± 0.22	h/H	1.24 ± 0.04	2.00 ± 0.07**
MUFA	34.86	28.53 ***	EPA	0.040±0.005	0.089 ± 0.01***
C 18:3 n-6	0.05 ± 0.01	1.00 ± 0.12***	DHA	0.248 ± 0.03	0.311 ± 0.020**
C 18:2 n-6	11.29 ± 0.97	4.12 ± 0.70***	EPA+DHA	0.288 ± 0.030	0.400 ± 0.032***

Statistical significance of differences between seasons (*** $p<0.001$; ** $p<0.01$; * $p<0.05$); SFA - Saturated Fatty Acid; MUFA - Monounsaturated Fatty Acid; PUFA-Polyunsaturated Fatty Acid; AI - Atherogenicity Index; TI - Thrombogenicity Index; h/H - Hypocholesterolaemic Index; EPA - eicosapentaenoic acid (C20:5n-3); DHA - docosahexaenoic acid (C22:6n-3)

Saturated and monounsaturated fatty acids

The round goby edible tissues presented high concentrations of three quantitatively dominating SFA: palmitic (C16:0), stearic (C18:0) and myristic (C14:0) acids which followed C16:0>C18:0>C14:0 distributions regardless of the seasons. The dominance of SFA group in the autumn goby was mainly due to the increase of C14:0, C18:0 and very long-chain FA C20:0-C24:0 ($p<0.001$). The amounts of MUFA's vary significantly between the seasons ($p<0.001$). The spring goby contained higher MUFA amount due to

higher levels of palmitoleic acid (C16:1 n-7) which is a 42.2 % from total MUFA's followed by oleic acid (C18:1n-9). This FA distribution significantly changed in autumn season (C18:1n-9>C16:1n-7), when both MUFAs levels significantly decreased - with 16 % (18:1n-9, $p<0.01$) and 53 % (16:1n-7, $p<0.001$) respectively.

Polyunsaturated fatty acids

Differences in the contents of individual PUFAs have been found between the two studied seasons. The important long-chain PUFAs such as eicosapentaenoic acid

(EPA, C20:5 n3), docosahexaenoic acid (DHA, C22:6 n-3), linoleic acid (LA, C18:2 n-6), arachidonic acid (ARA, C20:4 n-6) and alpha-linoleic (ALA, C18:3n-3) FA have been found in significant levels. The prevalent PUFA in the goby tissues was DHA regardless of the seasons. The highest obtained DHA values were in the spring goby (48.8 % of total PUFA), whereas in the autumn goby its value significantly decreased (up to 35.35 % of total PUFA, $p < 0.001$). Significant increase in autumn seasons has been found for EPA (up to 10 % of total PUFA, $p < 0.001$). In addition, in both seasons EPA levels have found lower, compared to ARA levels ($p < 0.001$). The round goby edible tissues were characterized by higher levels of n-3 PUFAs (56.35 % - 59.0 % of total PUFA) compared to n-6 PUFA levels (39.6 % - 43.4 % of total PUFA) in both seasons. The data presented in Table 3 indicated that the spring goby was characterized by higher level of n-3 PUFAs and lower levels of AI, TI and h/H indices. The

determined PUFA/SFA ratio has found higher than recommended value of 0.45 in both seasons (Table 3). This ratio decreased insignificantly in autumn samples ($p < 0.05$). With regard to the quality indices considered, all three indices (AI, TI and h/H) have showed significant statistical differences ($p < 0.001$) between seasons. AI and TI levels decreased in autumn samples, due to lower C16:0 values, which have high proatherogenic potential, whereas h/H index has showed opposite trend. The determined amounts of EPA+DHA showed significant seasonal differences ($p < 0.001$) and in 100g w.w. edible tissues their content ranged between 0.288g to 0.400g

Fat soluble vitamins content

The seasonal changes of vitamin contents and percentage of the daily recommended intake (RDI) of fat soluble vitamins in goby edible tissues are presented in Table 4.

Table 4: Total content and percentage of the daily recommended intake of fat soluble vitamins in goby edible tissues (mean±SD).

Analyte	Total content ($\mu\text{g } 100\text{g}^{-1} \text{ w. w.}$)		RDI (%)	
	Spring	Autumn	Spring	Autumn
All-trans-Retinol	0.9 ± 0.1	$14.8 \pm 0.7^{***}$	0.1 %	2.0 %
Cholecalciferol	4.9 ± 0.3	$2.6 \pm 0.2^{***}$	98.0 %	52.0 %
Alpha-Tocopherol	308.0 ± 23.4	$614.9 \pm 40.3^{***}$	2.0 %	4.1 %

SD-standard deviation; Statistical significance of differences between seasons ($^{***} p < 0.001$)

Significant differences in fat soluble vitamins ($p < 0.001$) between seasons have been recognised. Increases of retinol and alpha-tocopherol contents ($p < 0.001$) and significant reduction of vitamin D₃ content has been found in the autumn goby tissue, compared to spring season levels. The observed changes in vitamin A and E

content in this study showed a positive correlation with TL quantity. However, the reported results for vitamin A and E amounts, confirm that round goby is a poor source of these fat soluble vitamins. There are dietary standards in Bulgaria for relative daily intake (RDI) of fat soluble vitamins, which are in accordance with the

European Union standards with the exception of those for vitamin D₃ (5 µg for adults per day in Bulgaria against 10 µg per day in the European Union) (Ordinance No 23, 2005; DRI, 2011). The fat soluble vitamins provided by 100 g raw fish tissues as a percentage of the average daily allowance are compared with the RDI values for all-trans retinol, cholecalciferol and alpha-tocopherol amounts (see Table 4).

Discussion

In fish edible tissues, water, proteins and lipids make up about 98 % of the total mass, and the other minor constituents include carbohydrates, vitamins and minerals. The proportions of the constituents are species-specific and the main variations in the proximate composition between species and seasons occurs in moisture and lipid content (FAO, 2010; Prato and Biandolino, 2012; Stancheva *et al.*, 2013). This statement may confirm the result, presented in this study. Also this information is important to consumers and manufacturers regarding different aspects, like nutritional value or consideration regarding processing. Moreover, demersal fish species (e.g. Gobies) are usually low in lipid content and energy levels (kJ 100g⁻¹ raw tissue). On the other hand, the temperature increase strongly affects fat content of temperature-tolerated goby (Vassilev *et al.*, 2011). Therefore, the TL content often used as an indicator of food supply and reflects the special biochemical and ecological conditions of the marine environment. In this study, TL content showed significant seasonal differences ($p < 0.001$) which correlated with temperature variation and

goby considered in both seasons could be classified as a low-fat species (Table 2). In addition, the presented results are in good agreement with Black Sea goby species biology: mass spawning (during spring – April) - lowest TL (1.6 g 100g⁻¹w.w.) and intensive growth and fattening (during autumn – September) – higher TL content (2.65 g 100g⁻¹ w.w.) (Vassilev *et al.*, 2011). Earlier studies have been reported similar results for TL content for the round goby from South Eastern part of the Black Sea (Guner *et al.*, 1998; Tanakol *et al.*, 1999) and in our previously investigations for goby (Stancheva *et al.*, 2013) and for Black Sea turbot (Hadzhinikolova *et al.*, 2015). The determined protein content of *N. melanostomus* was at relatively high level throughout the year. Guner *et al.* (1998) has presented lower protein values for goby (15.40 g 100g⁻¹ w.w.) from the South Eastern part of the Black Sea. Our previous investigation has showed similar results for protein level and energy content of round goby (Stancheva *et al.*, 2013). Hadzhinikolova *et al.* (2015) has reported similar results for protein content (average 18.67 g 100g⁻¹ w.w.) for demersal Black Sea turbot. No comparable study is available for seasonal changes of Black Sea goby proximate composition.

Fatty acids composition

According to Tocher (2003), the SFA and MUFA contents in fish tissues may be result of a lipidic diet. Moreover, in addition to the diet, the SFA may be generated through non-lipidic carbon sources and then transformed into mono-unsaturated fatty acids. The observed domination of C16:0 and increase of C18:0 levels in SFA group in the autumn samples

can be explained by metabolic differences due to increase of seawater temperature, because these fatty acids are not usually subject to differences in diet. Kocatepe and Turan (2012) and Guizani and Moujahed (2015) reported similar SFA distribution (C16:0>C18:0>C14:0) for demersal red mullet and scorpion fish from Sinop region of the Black Sea, and Atlantic horse mackerel from the Tunisian Northern-East coast. In this study the amounts of MUFA's vary significantly between the seasons ($p<0.001$). Prato and Biandolino (2012) reported that the lean species such as black and grass goby from Mar Grande Sea, Italy, contained low level of C18:1 n-9, but other demersal species, such as red mullet, presented higher values of C 16:1 n-7. Some MUFAs are biomarkers for specific groups of hydrobionts: C18:1n-9 is used as a biomarker of dinoflagellates and bacterioplankton and C16:1n-7 is as biomarker of diatom microalgae (Murzina *et al.*, 2013; Vereshchaka and Anokhina, 2014). The observation of two times lower levels of C16:1n-7 in autumn season ($p<0.001$) can be explained with these facts that the phytoplankton bloom periods finish and water diatoms algae disappears. Many authors have reported that C18:1n-9 FAs are the most important lipids for cold-water organisms with respect to their adaptation to temperature and depth and this is a possible reason for its different levels observed in goby tissues in both seasons. In addition, the observed increase of monoenoic C20-C22 FA in autumn samples, may suggested the increase of preferred food levels (C20-22 are biomarkers of marine zooplankton, such as copepods) in this season. Physiological importance of these fatty acids for humans

is unclear and they are regarded as biologically inactive (Gladyshev *et al.*, 2012; Murzina *et al.*, 2013).

Among other ecological factors (such as water temperature, salinity) the qualitative content of food sources has been emphasized as a principal factor that defines the PUFA level in the tissues of fishes. Higher temperature (in autumn season) leads to decreased proportion of unsaturated FAs, such as DHA (Tocher, 2003). This statement is clearly confirmed by seasonal decrease of DHA/EPA and C16:0/C18:1n-9 ratios. According to Murzina *et al.* (2013) these ratios have indicated a lower degree of intensity of lipid metabolism, which is influenced on higher environmental temperature. The round goby quality have well demonstrated by higher levels of n-3 PUFAs compared to n-6 PUFA levels in both seasons. It is known that n-6 PUFAs may be proinflammatory and prothrombotic, and lower unchanged levels of this FA group found in both seasons may be advantageous to consumers for cardiovascular health. This finding can be explained by the observation that demersal species feed phytoplankton which contains low levels of n-6 FAs (Prato and Biandolino, 2012). Prato and Biandolino, (2012) presented similar high n-3 PUFA levels for black and grass goby species from Mar Grande Sea, Italy. In addition, n-6/n-3 ratio is very important for human intake as both of them are in competition to the same enzyme to synthesize prostaglandins derived from both n-3 and n-6 families. Simopoulos (2013) suggested that a decrease in the human dietary n-6/n-3 PUFA ratio is essential to help prevent coronary heart disease by reducing the plasma lipids and

to reduce the risk of cancer. PUFA/SFA ratio is always used for assessment of fish nutrition quality. Several studies have been found inverse correlation between this ratio and cardiovascular diseases and suggested that the replacement of SFA with PUFA in the human diet will decrease similar health problems (Simopoulos, 2013). Department of Health (1994) recommended values of PUFA/SFA ratio greater than 0.45. This cut-off value of cited ratio coincided with information of the English Health Department: Foods with ratio under 0.45 have the potential to raise blood cholesterol levels. The nutritional value of round goby also was determined by lipid quality indices. These indices well described the functional properties of fish lipids. With regard to the quality indices considered, all three indices (AI, TI and h/H) showed significant differences ($p < 0.001$) between seasons. Higher values of AI and TI (> 1.0) are detrimental to human health, whereas higher h/H levels ($> 1.0 \pm 0.2$) have been recommended. The observed well balanced PUFA/SFA ratio, higher level of n-3 PUFAs and h/H index; lower levels of AI and TI indices found in autumn season also could be explained by the type of food available and the abiotic environmental factors. Thus the reported levels showed the high quality of Black Sea round goby lipids especially of autumn seasons. To our knowledge, in literature there is no data describing seasonal changes in FAs ratios and lipid quality indices for the round goby caught from the Black Sea waters. In additions, the European Food Safety Authority (EFSA, 2012) recommended daily intake (RDI) of 0.250 to 0.500 g EPA+DHA for healthier people. A 200 g of edible tissues of the goby contained from

0.576 g to 0.800 g of EPA+DHA n-3 PUFA (depending on the season) and provides 115 %-160 % of EPA+DHA n-3 PUFA RDI. The result of this study shows that the Black Sea goby is a very good source of n-3 PUFAs which are known to be of especial physiological importance for consumer's health, during the year.

Fat soluble vitamins

Several studies have reported that vitamin D content was 4 - to 9-fold higher in wild than in cultured fish (Ruxton and Derbyshire, 2009; Ohrvik *et al.*, 2012). Oily fishes are rich in vitamin D, containing 2.6-16.1 mg 100g⁻¹. Presented results are in the range in both seasons. Earlier investigations of the goby (Stancheva *et al.*, 2012) showed lower values for vitamin A content. The fish food (copepods, decapods and other) contain relatively large quantities of vitamin A. The greater rise in the marine phyto- and zooplankton begins as soon as the intensity and duration of sunlight increase during the spring-autumn period in the Black Sea. Current investigation of composition and dynamics of the Black Sea benthopelagic plankton reported that its share decreased in the progression: Winter-spring-summer-autumn, due to increasing growth and reproduction of the fish species, which well correlated with presented results for Vitamins A and E (Vereshchaka and Anokhina, 2014). According to the Bulgarian dietary standards for average daily intake of fat soluble vitamins, the goby showed low percentages for the RDI of retinol and alpha-tocopherol regardless of the seasons, whereas the cholecalciferol content supplied human needs up to 100 % RDI and we can classify the Black Sea

goby as a very good source of vitamin D₃ in all year.

Present study was carried out to find out the seasonal changes of nutritional quality of economically important round goby caught from the Bulgarian part of the Black Sea. Results have showed that investigated species is a valuable food source of high quality protein, with low lipid content and a well-balanced fatty acids composition. Many researchers believed most of fatty fishes are a great sources of long-chain n-3 PUFA. However, in this study we found out that lean fish such as round goby contained up to 0.400 g EPA+DHA in 100 g edible tissues which is around 160% of RDI. In addition, the high levels of Vitamin D₃ content enhance the quality of round goby as a food. It could be concluded that the round goby populations are rich source of n-3 PUFA and Vitamin D₃. Hence, regular consumption of the Black Sea goby is highly recommended since this fish is an excellent source of valuable nutritional components regardless of the seasons. Given the lack of existing data on seasonal changes in nutrient composition of round goby species from Black Sea, the presented results will contribute to the current knowledge and the better understanding the FAs composition of food objects and the ability of fish to modify the composition of ingested FAs with regard to ambient conditions.

Acknowledgments

The authors acknowledge the facilities provided and financial supported by the Faculty of Pharmacy, Medical University of Varna for the study.

References

- AOAC, 1990.** Official methods of analysis of the Association of Official Analytical Chemists. 15th edition. Washington, DC, Association of Official Analytical Chemists. 771 P.
- AOAC 950.46, 2000.** Moisture in meat and meat products. Air Drying Method, AOAC Official methods of Analysis, 17th ed. Association of Official Analytical Chemists. Gaithersburg, MD, USA. 2200 P.
- BDS 9374: 1982.** Meat and meat quality. Determination of crude protein in meat. 25 P (Bg).
- BDS EN ISO 12966-4: 2015.** Animal and vegetable fats and oils-Gas chromatography of fatty acid methyl esters-Part 4: Determination by capillary gas chromatography. 30 P.
- BDS EN ISO 12966-2: 2017.** Animal and vegetable fats and oils-Gas chromatography of fatty acid methyl esters-Part 2: Preparation of methyl esters of fatty acids. 37 P.
- Bligh, E. and Dyer, W.J., 1959.** A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), 911–917.
- Boran, G. and Karaçam, H., 2011.** Seasonal Changes in proximate composition of some fish species from the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, 11(1), 01-05.
- Commission Regulation (EU), No. 116. 2010.** of 9 February 2010 amending Regulation (EC) No 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims. 16 P.

- Creteanu, M.D., Banaru, N., Papadopol, D., Crasmaru, M. and Vasilescu, G., 2008.** Biochemistry and heavy metal values in some Gobidae species from the Romanian Black Sea coast. *Cercetari Marine*, 38, 325–336.
- Department of Health 1994.** Nutritional aspects of cardiovascular disease: Health and social subjects (Report No 46) HMSO, London. 187 P.
- Dietary Reference Intakes (DRI) for Calcium and Vitamin D., 2011.** from <https://www.ncbi.nlm.nih.gov/books/NBK56070/>
- Dobreva, D.A., Galunska, B. and Stancheva, M., 2011.** Liquid chromatography method for the simultaneous quantification of fat soluble vitamins in fish tissue. *Scripta Scientifica Medica*, 43(1), 276–279.
- European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA), 2012.** Scientific opinion on the tolerable upper intake level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). *EFSA Journal*, 10, 2815–2863.
- FAO, 2010.** Nutrition elements of fish. The state of fisheries and aquaculture. Rome, 2010. 218 P.
- FAO/INFOODS Guidelines., 2012.** FAO/INFOODS Guidelines for converting units. Retrieved from <http://www.fao.org/docrep/017/ap796e/ap796e.pdf>
- Ghomi, M.R., Von Elert, E., Borcharding, J. and Fink, P., 2014.** Fatty acid composition and content of round goby (*Neogobius melanostomus* Pallas 1814) and monkey goby (*Neogobius fluviatilis* Pallas 1814), two invasive gobiid species in the lower Rhine River (Germany). *Journal of Applied Ichthyology*, 30(3), 527-531.
- Gladyshev, M., Lepskaya, E., Sushchik, N., Makhutova, O.N., Kalachova, G.S., Malyshevskaya, K.K. and Markevich, G.N., 2012.** Comparison of polyunsaturated fatty acids content in filets of Anadromous and Landlocked Sockeye Salmon (*Oncorhynchus Nerka*). *Journal of Food Sciences*, 77(12), C1307-C1310.
- Guizani, S. and Moujahed, N., 2015.** Seasonal variation of chemical and fatty acids composition in Atlantic Mackerel from the Tunisian Northern East Coast. *Journal of Food Process Technology*, 6(9), 1-4.
- Guner, S., Dincer, B., Alemdag, N. and Colak, A., 1998.** Proximate composition and selected mineral content of commercially important fish species from the Black Sea. *Journal of Sciences and Agriculture*, 78(3), 337–342.
- Hadzhinikolova, L., Ivanova, A. and Hubenova, T., 2015.** Chemical and fatty acid composition of turbot (*Psetta maxima maeotica*) meat from the Bulgarian Black Sea shores, Proceeding of VII International Conference “Water and fish”. Beograd, Serbia, COBISS.SR. 572 P.
- Health of the Nation Report, 2009.** Retrieved from: <http://ncpha.government.bg/files/Doklad%20Zdrave%20na%20naciata%202009.pdf>
- Jump, D.B., Depner, C.M. and Tripathy, S., 2012.** Omega-3 fatty acid supplementation and cardiovascular

- disease. *Journal of Lipid Research*, 53, 2525-2545.
- Kocatepe, D. and Turan, H., 2012.** Proximate and fatty acid composition of some commercially important fish species from the Sinop region of the Black Sea. *Lipids*, 47(6), 635-641. DOI:10.1007/s11745-012-3658-1
- Murzina, A.S., Nefedova, Z.A., Nemova, N.N., Ripatti, P.O. and Pekkoeva, S.N., 2013.** Specific fatty acid status in the White Sea herring from different bays in the White Sea in regard to ecological factors. In BIONATURE. The Fourth International Conference on Bioenvironment, Biodiversity and Renewable Energies. pp. 9-12.
- National Strategic Plan for Fisheries and Aquaculture. 2007–2013.** Ministry of agriculture and forestry. National Agency for Fisheries and Aquaculture. 78 P.
- Ohrvik, V., Malmborg, A., Mattisson, I., Wretling, S. and Astrand C., 2012.** Fish, shellfish and fish products - analysis of nutrients, (Report No.1). Sweden. Livsmedels Verket. 48 P.
- Ordinance No 23/19.07.2005.** On the physiological feeding of population. <http://www.lex.bg/laws/ldec/213550842> 8
- Prato, E. and Biandolino, F., 2012.** Total lipid content and fatty acid composition of commercially important fish species from the Mediterranean, Mar Grande Sea. *Food Chemistry*, 131(4), 1233-1239.
- REGULATION (EC) No 1924, 2006,** Of the European parliament and of the council of 20 December 2006 on nutrition and health claims made on foods. 17 P.
- http://www.fsai.ie/uploadedFiles/Cor_Reg1924_2006.pdf
- Ruxton, C. and Derbyshire, E., 2009.** Health impact of vitamin D: Are we getting enough? British Nutrition Foundation. *Nutrition Bulletin*, 34(2), 185–197.
- Santos-Silva, J., Bessa, R.J.B. and Santos-Silva, F., 2002.** Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. *Livestock Production Sciences*, 77(3-4), 187-194.
- Simopoulos, A., 2013.** Dietary omega-3 fatty acid deficiency and high fructose intake in the development of metabolic syndrome, brain metabolic abnormalities, and non-alcoholic fatty liver disease. *Nutrients*, 5(8), 2901–2923.
- Stancheva, M., Galunska, B., Dobрева, D.A. and Merdzhanova, A., 2012.** Retinol, alpha-tocopherol and fatty acid content in Bulgarian Black Sea fish species. *Grasas y aceites*, 63(2), 152-157.
- Stancheva, M., Merdzhanova, A., Petrova, E. and Petrova, D., 2013.** Heavy metals and proximate composition of Black Sea Sprat (*Sprattus sprattus*) and Goby (*Neogobius melanostomus*). *Bulgarian Journal of Agricultural Science*, 19(1), 35-41.
- Stark, K.D., Van Elswyk, M.E., Higgins, M.R., Weatherford, C.A. and Salem, N., 2016.** Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. *Progress in Lipid Research*, 63, 132-152.

- Tanakol, R., Yazici, Z., Sener, E. and Sencer, E., 1999.** Fatty acid composition of 19 species of fish from the Black Sea and Marmara Sea. *Lipids*, 34(3), 291–294.
- Tocher, D.R., 2003.** Metabolism and functions of lipids and fatty acids in teleost fish. *Review Fish Sciences*, 11(2), 107–184.
- Ulbricht, T.L.V. and Southgate, D.A.T., 1991.** Coronary heart disease: Seven dietary factors. *Lancet*, 338, 985-992.
- Vassilev, M., Apostolou, A., Velkov, B., Ivanova, P., Panayotova, M., Dobrev, D. and Pehlivanov, L., 2011.** Status of gobiid ichthyofauna in Bulgaria: Taxonomical, conservative and social aspects. *Natura Montenegrina, Podgorica*, 10(2), 115-124.
- Vereshchaka, A.L. and Anokhina, L.L., 2014.** Composition and dynamics of the Black Sea benthopelagic plankton and its contribution to the near-shore plankton communities. *PLoS ONE*, 9(6), e99595.