PAPER

INFLUENCE OF SEASON AND FARMING LOCATION ON THE QUALITY PARAMETERS OF SEA BASS (*DICENTRARCHUS LABRAX*) AND SEA BREAM (*SPARUS AURATA*)

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ABSTRACT

The study determined chemical composition of sea brass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) farmed in Adriatic Sea, together with variation caused by seasonal variations and farming location. Samples were collected from four different fish farms at three times: June 2012, October 2012 and January 2013. The presented results clearly show seasonal variations of moisture and fat content in the edible part of the fish, while the farming location was proven not to have any significant impact ($p>0.05$). Fatty acid composition was significantly influenced both by the season and the farming location ($p<0.05$). The resulting n-3/n-6 ratios were lower than those reported in other studies, which can be attributed to differences in diet the fish were fed on. Seasonal variations and farming location did not affect fish mineral composition, but mutual differences between the two species were significant.

- Keywords: Sea bass, Sea bream, Quality parameters, Season, Farming location -

INTRODUCTION

European sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) are some of the most important finfish species farmed in the Mediterranean region (GRIGORAKIS *et al*., 2002; ALASALVAR *et al*., 2002; KYRANA and LOUGOVOIS, 2002; FUENTES *et al*., 2010). Fish as human seafood is widely consumed because of its high nutritional value, i.e. high protein content, low saturated fatty acid content and high n-3 fatty acid content. Studies have reported that the consumption of only one fatty fish meal per day can result in an approximate n-3 fatty acid intake of 900 mg/day (EPA and DHA), sufficient to have a protective effect on cardiovascular system (KRIS-ETHERTON *et al*., 2002). Furthermore, fish is a rich source of minerals (e.g. sodium, calcium, iron, magnesium, copper, zinc and selenium) and vitamins (e.g. vitamin A, E and D) (FAO/WHO, 2002; CAPELLI *et al*., 2008; CUSTÓDIO *et al*., 2011).

Organoleptic properties and nutritional value are two sets of characteristics that, together with freshness, are accountable for fish quality. Both of them strongly depend on the chemical composition of fish and are affected by many factors including intrinsic fish characteristics (such as species, age, sex, etc.), environmental factors (seasonal changes in temperature, salinity, etc.) and feeding regimen (composition of the feed in use, feeding ratio, etc.) (PIRINI *et al*., 2000; CORDIER *et al*., 2002; GRIGORAKIS *et al*., 2004; GRIGORAKIS, 2007).

The quality of farmed fish strongly depends on factors involved into the production processes (PERIAGO *et al*., 2005). Studies have also shown that, like most Mediterranean fish, farmed fish spawns between December and March, correspondent to the peak sex steroid levels in their plasma. This period is featured by ovarian growth and a substantial reduction in food intake (CORDIER *et al*., 2002; CERDÀ *et al*., 1995). Feeding on commercial nutritive products directly influences growth rates and meat quality, especially its lipid content and composition that can both be modified by diet (IZQUIERDO *et al*., 2005). At the same time, an intense production of cultured fish has raised concerns over the quality of such fish in comparison to the wild one (ALASALVAR *et al*., 2002). An advantage of farmed fish over a wild-caught is that the first is produced and harvested under controlled conditions, so that hazards associated with fish consumption get to be reduced.

A number of previous studies dealing with physico-chemical and organoleptic properties of wild and cultivated sea bream and sea bass have drawn attention to differences in fish quality, especially to the differences in lipid content and saturated and unsaturated fatty acid composition (GRIGORAKIS *et al*., 2002; ALASALVAR *et al*., 2002; FUENTES *et al*., 2010; CUSTÓDIO *et al*., 2011; PERIAGO *et al*., 2005; SAGLIK *et al*., 2003; ORBAN *et al*., 2003). However, no data on the quality of these two species cultivated in the area of the Eastern Adriatic coast under our study, nor data on possible differences in fish meat composition between the two, arising from farming locations or seasonal variations, have been published insofar. Therefore, the aim of this study was to evaluate the influence of season and geographical location on quality parameters of farmed sea bass and sea bream by virtue of analysing their chemical parameters, mineral and fatty acid composition.

MATERIALS AND METHODS

Growth conditions and fish sampling

Three individual samples of market size fish containing ten specimens of sea bass with mean body weight 342.77±86.90 g (92.92-473.18 g) and sea bream with mean body weight 294.70± 49.46 g (184.85-351.35 g) were collected in June 2012, October 2012 and January 2013 from four different commercial marine fish farms. Two of these farms (1 & 2) are situated in the northern part of the Adriatic coast (Istria), while the remaining two (3 & 4) are situated in the mid-eastern coast (Dalmatia). Despite of their common location on the eastern Adriatic coast, each of these sites has its particularities. Site 1 is situated in a very long bay protected from the influence of an open sea, unlike Site 2, which is situated at the entrance of the bay and is exposed to currents. At both sites, the temperature of the sea water fluctuates from 6°C in the winter to 2°C in the summer due to water shallowness. The remaining two sites are situated in the mid-coast, on the islands exposed to currents and waves, so that the sea water temperature fluctuates less strikingly. Its lowest temperature registered in the winter is about 12°C, while its highest temperature registered in the summer is about 25°C. Fry is referred to on-growing originated from different commercial hatcheries and was cultivated in floating net cages of different size and shape for 22 to 28 months, the cultivation density thereby ranging from 5 to 12 kg per cubic meter. The fry was fed on a commercially available fish feed, with a daily ratio of 4.0-1.5 % of body weight for juveniles and 1.5-0.6% of body weight for premarket size. Daily ratio was dependent on the sea temperature, photoperiod and oxygen saturation according to manufacturer's recommendations. All samples of sea bass and sea bream were fed on a same commercial feed manufactured by international feed mills. According to the manufacturer's declaration, the feed on which the sea bass was fed contained 40% of proteins, 24% of fat, 3% of fibres, 6.3% of ash and 0.9% of phosphorus from fish meal, as well as soybean meal, corn gluten, wheat and

wheat by-products. The composition of the feed on which the sea bream was fed was very similar, with slight differences in the share of proteins (43%) and fat (16%).

The fish was killed by virtue of immersing into ice/water slurry and transported to the laboratory on ice. Before eviscerating and filleting the edible part of the fish for analysis, body weight and body length were measured to the closest g (184.85 to 473.18 g) and cm (23.9 to 31.7 cm).

Analysis of compositional parameters

Ten fillets per individual sample were homogenized separately using Grindomix GM200 (Retch, Germany), so as to obtain a homogeneous sample for the determination of chemical parameters. For the sake of analysis, the entire meat portion was employed as edible. The sea bass and sea bream samples were analysed using standard analytical methods: ISO 1442:1997 (moisture), ISO 936:1998 (ash), ISO 937:1978 (crude protein) and ISO 3496:1994 (hydroxyproline/collagen). Sodium and calcium content were determined by using an in-house-validated titration methods described by TRAJKOVIĆ *et al.* (1983). For the determination of sodium, 2 g of the sample were homogenized with sand and 3 mL of water, transferred into a 100 mL-volumetric flask, stirred and placed into a water bath at 100 °C for 15 min. After cooling, the mixture was made up to volume with water and filtered. An aliquot (25 mL) of the filtrate was transferred into an Erlenmeyer flask containing a few drops of K_2CrO_4 (62 g/100 mL of water) as indicator and titrated with 0.1 M-$AgNO_3$ until a persistent reddish colour was obtained. Sodium content was calculated based on the volume of titration reagent used and its concentration. For the determination of calcium, the sample was heated in a furnace at 550 °C up to white ashes were obtained. After cooling, ashes were transferred into a 250 mL-glass, into which 40 mL of HCl (30%), 60 mL of water and few drops of HNO_3 (65%) were added. The solution was boiled for 30 min, cooled, and transferred into a 250 mL-volumetric flask and filled with water up to the mark. An aliquot of the filtered solution (100 mL) was transferred into a 250 mL-glass into which 1 mL of citric acid (300 g/L) and 5 mL of ammonium chloride solution (50 g/L) were added and made up to 100 mL with water. The solution was shortly boiled; then, 10 drops of bromocresol green solution and 30 mL of hot ammonium oxalate solution (4,5 g/100 mL of water) were added. The solution was neutralized by the addition of ammonia (25%) with constant steering up to pH 4.4-4.6, when the colour of the solution turned light blue. The solution was then left in a dark place for 30 min and filtered. After addition of 80 mL of H_2SO_4 (20%), the solution was heated to 80 °C till all precipitates were dissolved and then titrated with $KMnO_4$ (0.1 N) till the pink colour of the solution remained stable for 1 min. Calcium content was calculated based on the expenditure of titration reagent and its concentration.

For the determination of phosphorus, ISO 13730:1996 spectrophotometric method was employed. All chemicals used for the analyses were of an analytical grade.

Lipid analysis

Total lipid contents were determined gravimetrically after extraction in a Soxhlet apparatus according to the AOAC method 948.22:2000. Homogenized samples (5 g) had been extracted with petrol ether for six hours. The solvent was evaporated to dryness in a heated oven at 105°C, following which total lipid content was calculated.

For fatty acid analyses, extracted triacylglycerols were converted into corresponding fatty acid methyl esters (FAME) by trans-esterification with methanolic solution of potassium hydroxide. Approximately 60 mg of the sample was weighed into a test tube equipped with a glass stopper, and dissolved in 4 mL of isooctane. After that, 200 μL of potassium hydroxide solution in methanol (2 mol/L) was added; the reaction was carried out at the room temperature, enhanced by vigorous shaking (2 x 30 s). The solution was neutralized by adding 1 g of sodium hydrogen sulphate monohydrate and transferred into a 2 mL vial. GC analyses were performed on CP-3800 (Varian, Palo Alto, USA) using split/splitless injector and flame-ionisation detector. Capillary column DB 23ms 60 m x 0.25 mm, film thickness 0.25 μm, was used, the temperature thereby being first set at 60°C, then risen up to 210°C at the rate of 4 °C/min, and then rested at 210°C for 15 minutes. Helium was used as a carrier gas at a flow rate of 1 mL/min. The temperature of the split/splitless injector was 250 °C; the same applies to the flame-ionisation detector, while the split ratio equalled to 1:20. The samples were injected manually (1.0 μL). The detector flow rates were as follows: hydrogen 30 mL/min, air 300 mL/min, and detector makeup gas was helium with flow rate of 27 mL/min. A detailed description of the employed method and its suitability for the given purpose was published elsewhere (PETROVIĆ *et al.*, 2010).

Statistical analysis

The statistical significance of difference between samples was tested by analysis of variance (ANOVA) using Statistica Ver. 10.0 Software (STATSOFT INC. 1984-2011, USA). A p-value of 0.05 was considered statistical significance (p=0.05).

RESULTS AND DISCUSSION

Mean values and standard deviations of chemical parameters determined in farmed sea bass and sea bream sampled at different Adriatic coast sites and at three different times, are shown in Table 1 and Table 2, respectively. A significantly higher total fat content was observed for sea bream in comparison to sea bass, with a significant rise in total fat content in both species sampled in October in comparison to those sampled in June and January. Results of our study also showed the same proportion of fat and moisture in both species, with fat content in range from 3.2 to 12.3% in sea bass and 4.2 to 15.0% in sea bream, dependent on, and highly varying according to, the farming season. Our results respective to the seasonal variations of seam beam's fat content are in agreement with the results of CARDINAL *et al.* (2011).

Previous data have shown the approximate composition of sea bass to be 70.71% of moisture, 20.35% of protein, 6.10% of total fat and 1.66% of ash (ERKAN and ÖZDEN, 2007) or, according to another source, 76.72% of moisture, 19.43% of protein, 4.81 % of total fat and 1.23% of ash (KYRANA and LOUGOVOIS, 2002); the aforementioned data are similar to the results of this study obtained for the sea bass sampled in June and January. In 2001, HUIDOBRO *et al.* reported the chemical composition of the Spanish sea bream to be 71.83% of moisture, 22.31% of protein, 5.28% of total fat and 1.27% of ash, whereas ALASALVAR *et al.* (2002) gave the approximate composition of 74.74% of moisture, 18.80% of protein, 6.53% of fat and 1.53% of ash. These results are also comparable to the values obtained in this study for the sea bream sampled in June.

As fish is included in the category of ectothermic poikilotherms, the content of fat in a certain period of the year can be explained by physiological process of fat stock saving or spending. High values of fat obtained in October for all sampling sites and in both species, indicate the preparation of fish bodies for the winter period that comes after a long period of intense feeding. Reduction of fat content in the winter mirrors the spending of fat consequent to the diet minimized due to the low sea temperature and slow fish metabolism. In early summer, when a change in the environmental conditions occurs, primarily in terms of temperature rises, fish metabolism is accelerated, and the energy taken from the food is used for fish growth, which, as seen in both this and earlier studies (JAMES, 1995), resulted in a lower fat content.

The analysis of variance (ANOVA) revealed statistically significant differences ($p<0.05$) in moisture, fat and crude protein content of the sea bass, and statistically significant differences in moisture and fat content in the sea bream dependent on the sampling season (Ta-

Table 1 - Mean chemical composition (±SD) of the sea bass.

	June 2012			October 2012			January 2013		
Parameter	**Farm 1**	**Farm 2**	**Farm 3**	**Farm 1**	**Farm 2**	**Farm 3**	**Farm 1**	**Farm 2**	**Farm 3**
Moisture (%)	73.2±2.4	74.0±1.8	75.5±2.3	67.5±1.6	72.0±2.6	68.8±2.28	70.9±2.87	72.4±2.01	72.6±1.85
Ash (%)	1.16±0.13	1.24±0.15	1.42±0.07	1.32±0.12	1.40±0.09	1.27±0.10	1.62±0.11	1.44±0.06	1.40±0.09
Fat (%)	6.6±0.3	3.7±0.4	3.2±0.2	12.3±1.6	8.0±1.1	11.9±0.9	6.0±0.6	5.5±0.4	5.1±0.2
Crude protein (%)	20.64±0.82	21.50±0.71	22.52±1.21	19.30±1.18	19.09±0.98	18.89±1.02	21.47±1.22	20.51±0.95	20.82±1.03
Hydroxyproline (%)	0.059±0.007	0.064±0.005	0.059±0.010	0.061±0.008	0.059±0.005	0.060±0.004	0.061±0.007	0.065±0.006	0.064±0.008
Collagen (%)	0.47±0.06	0.51±0.04	0.47±0.08	0.49±0.06	0.47±0.04	0.48±0.03	0.49±0.06	0.52±0.05	0.51±0.06
Ca (mg/kg)	701±12.1	685±11.4	731±9.2	754±12.3	813±17.1	662±18.4	723±20.5	802±21.8	725±24.3
P (mg/kg)	3665±33.6	3913±28.5	3843±17.5	3751±30.1	3656±26.5	3721±25.3	3616±41.3	3856±34.5	3589±29.4
Na (mg/kg)	626±7.5	621±9.3	568±7.3	515±6.6	711±10.4	625±8.6	638±7.1	558±6.9	738±8.9

Table 2 - Mean chemical composition (±SD) of sea bream.

	June 2012			October 2012			January 2013		
Parameter	**Farm 1**	**Farm 2**	**Farm 4**	**Farm 1**	**Farm 2**	**Farm 4**	**Farm 1**	**Farm 2**	**Farm 4**
Moisture (%)	72.7±2.1	71.1±1.7	74.5±1.3	66.2±1.8	64.3±2.0	66.5±1.8	67.2±1.7	68.3±2.1	66.3±2.4
Ash (%)	1.33±0.14	1.34±0.09	1.33±0.17	1.32±0.08	1.61±0.13	1.36±0.07	1.71±0.09	1.32±0.11	1.41±0.06
Fat (%)	6.9±0.15	7.4±0.22	4.2±0.17	13.5±0.26	15.0±0.34	14.2±0.28	10.9±0.17	11.3±0.29	11.6±0.22
Crude protein (%)	21.06±1.09	20.32±1.15	21.73±1.08	19.42±1.15	19.65±1.66	18.76±1.09	20.39±1.23	19.14±1.31	20.76±0.92
Hydroxyproline (%)	0.069±0.008	0.070±0.012	0.063±0.008	0.071±0.006	0.073±0.010	0.070±0.011	0.061±0.009	0.062±0.007	0.065±0.006
Collagen (%)	0.55±0.06	0.56±0.10	0.50±0.06	0.57±0.05	0.58±0.08	0.56±0.09	0.49±0.07	0.50±0.06	0.52±0.05
Ca (mg/kg)	281±5.6	255±8.7	246±9.1	268±7.4	303±5.6	241±3.8	269±4.5	307±5.2	261±3.9
P (mg/kg)	3551±19.6	3456±25.8	3503±22.7	3321±18.4	3410±25.7	3545±28.8	3412±16.3	3478±23.4	3388±17.6
Na (mg/kg)	312±4.7	365±3.4	311±6.2	268±2.5	338±3.8	341±4.5	283±5.1	298±3.9	319±4.8

Table 3 - Statistical analyses (ANOVA) of chemical parameters witnessed on various farming locations and during different farming seasons.

Parameter of analysis	Sea bass (p values)		Sea bream (p values)	
	Season	Location	Season	Location
Moisture	0.026*	0.193	0.008*	0.623
Ash	0.243	0.998	0.615	0.833
Fat	0.012*	0.207	0.002*	0.460
Crude protein	0.031*	0.814	0.090	0.485
Hydroxyproline	0.250	0.466	0.053	0.647
Collagen	0.250	0.534	0.059	0.609
Ca	0.583	0.431	0.558	0.154
P	0.472	0.442	0.512	0.785
Na	0.867	0.793	0.430	0.170

*significantly different ($p<0.05$).

ble 3). Farming location had no significant effect ($p>0.05$) on chemical parameters and mineral composition of either of the two. DEL COCO *et al.* (2009) studied the difference between the nutritional value of sea bream produced within three different farming systems and the wild fish. Their results showed a significant difference in protein, lipid and cholesterol content between the fish grown within different farming systems. Among fatty acids only oleic acid varied significantly.

Fatty acid composition, expressed as mean values and standard deviations obtained for sea bass and sea bream samples is shown in Table 4 and Table 5. The most represented fatty acid in both analysed species was oleic acid (C18:1 n-9, OA), followed by linoleic acid (C18:2 n-6, LA) and palmitic acid (C16:0, PA). STROBEL *et al.* (2012) have recently reviewed studies that

Table 4 - Fatty acid composition (%) of the sea bass[1].

	June			October			January		
Fatty acid	Farm 1	Farm 2	Farm 3	Farm 1	Farm 2	Farm 3	Farm 1	Farm 2	Farm 3
C14:0	3.53±0.24	2.56±0.16	3.71±0.21	2.92±0.18	3.34±0.09	2.85±0.10	4.05±0.36	2.51±0.15	3.84±0.16
C15:0	0.35±0.01	0.30±0.02	0.47±0.17	0.30±0.11	0.43±0.01	0.30±0.01	0.37±0.03	0.25±0.01	0.35±0.05
C16:0	16.97±0.12	15.49±0.35	16.61±0.39	15.11±0.38	18.23±0.35	15.60±0.68	16.37±0.27	15.74±0.42	16.74±0.44
C16:1n-7	4.45±0.06	3.77±0.14	5.28±0.29	3.64±0.19	3.63±0.06	3.77±0.14	4.30±0.15	3.31±0.41	4.97±0.26
C17:0	0.55±0.02	0.52±0.11	0.65±0.09	0.53±0.05	0.67±0.02	0.55±0.02	0.55±0.04	0.43±0.03	0.37±0.02
C17:1n-7	0.12±0.03	0.17±0.01	0.32±0.09	0.18±0.01	0.14±0.01	0.17±0.01	0.18±0.03	0.16±0.01	0.17±0.02
C18:0	3.55±0.27	3.86±0.22	3.87±0.11	3.64±0.11	5.07±0.22	3.82±0.25	2.94±0.04	4.12±0.20	3.96±0.18
C18:1n-9	25.53±0.37	26.70±0.19	26.68±0.23	30.31±0.47	31.84±0.54	31.16±0.21	24.38±0.33	29.50±0.58	21.86±0.36
C18:1n-7	2.59±0.14	2.36±0.06	2.66±0.23	2.54±0.08	2.71±0.09	2.61±0.10	3.07±0.22	1.92±0.32	2.76±0.12
C18:2n-6	18.16±0.37	25.81±0.34	20.92±0.29	22.30±1.19	16.01±0.34	21.66±0.91	16.49±0.57	23.83±0.69	21.49±0.28
C18:3n-6	0.08±0.01	0.10±0.02	0.28±0.01	0.19±0.07	0.21±0.04	0.18±0.01	0.22±0.03	0.22±0.01	0.20±0.02
C18:3n-3	2.69±0.11	3.22±0.14	3.21±0.23	5.61±0.19	2.71±0.04	4.42±0.68	3.04±0.11	4.49±0.71	2.67±0.18
C18:4n-3	0.90±0.03	0.57±0.05	0.76±0.02	0.56±0.02	0.46±0.01	0.51±0.07	1.23±0.02	0.53±0.03	0.88±0.23
C20:0	0.31±0.02	0.28±0.02	0.31±0.02	0.31±0.03	0.49±0.02	0.30±0.02	0.16±0.02	0.28±0.02	0.16±0.05
C20:1n-9	3.75±0.28	1.93±0.19	1.48±0.14	2.17±0.35	2.24±0.38	2.09±0.11	5.27±0.32	2.54±0.40	1.75±0.08
C20:2n-6	1.13±0.27	0.95±0.03	0.87±0.08	0.73±0.17	0.25±0.01	0.76±0.03	0.68±0.02	0.76±0.08	0.82±0.20
C20:3n-6	ND	ND	0.08±0.03	0.08±0.04	0.06±0.00	0.08±0.01	0.05±0.03	0.08±0.02	0.18±0.01
C20:4n-6	0.33±0.02	0.33±0.01	0.33±0.02	0.30±0.03	0.58±0.02	0.24±0.01	0.35±0.07	0.28±0.06	0.44±0.02
C20:3n-3	ND	ND	0.09±0.02	0.15±0.06	0.10±0.01	0.11±0.04	0.16±0.02	0.12±0.02	0.19±0.02
C20:4n-3	0.46±0.02	0.42±0.02	0.43±0.13	0.30±0.01	0.44±0.04	0.29±0.01	0.48±0.02	0.29±0.02	0.37±0.02
C22:0	ND	0.09±0.02	ND	0.15±0.01	0.29±0.12	0.16±0.02	0.07±0.01	0.16±0.01	0.32±0.03
C20:5n-3	3.46±0.90	3.64±0.54	2.88±0.14	2.61±0.35	2.18±0.15	2.72±0.15	3.78±0.44	2.55±0.27	6.65±0.29
C22:1n-11	3.24±0.15	1.04±0.09	0.86±0.39	1.03±0.05	1.57±0.23	0.93±0.14	3.53±0.32	1.16±0.42	0.60±0.06
C22:1n-9	0.38±0.02	0.20±0.01	0.39±0.02	0.26±0.03	0.46±0.01	0.24±0.03	0.45±0.02	0.26±0.03	0.29±0.10
C22:5n-3	0.92±0.14	0.80±0.22	1.81±0.16	0.71±0.16	0.71±0.05	0.71±0.02	1.00±0.32	0.70±0.21	1.70±0.14
C24:1n-9	0.41±0.03	0.24±0.02	0.40±0.08	0.20±0.12	0.71±0.02	0.21±0.03	0.23±0.05	0.17±0.01	0.13±0.03
C22:6n-3	6.14±0.21	4.65±0.34	4.66±0.22	3.21±0.55	4.49±0.72	3.58±0.53	6.58±0.23	3.62±0.42	6.15±0.20
SFA	25.26±0.19	23.09±0.64	25.61±0.88	22.96±0.29	28.52±0.64	23.56±0.97	24.52±0.32	23.49±0.48	25.70±0.97
MUFA	40.46±0.38	36.41±0.26	38.07±0.24	40.35±0.72	43.28±0.47	41.18±0.24	41.41±0.29	39.03±0.47	32.58±0.34
PUFA	34.28±0.57	40.50±0.74	36.32±0.75	36.69±0.91	28.20±0.68	35.25±1.21	34.06±0.41	37.48±0.58	41.72±0.62
Total n-3	14.58±0.40	13.30±0.11	13.85±0.70	13.14±0.62	11.09±0.29	12.34±1.02	16.27±0.37	12.30±0.20	18.60±0.54
Total n-6	19.70±0.37	27.20±0.48	22.47±0.35	23.55±0.35	17.11±0.48	22.91±0.76	17.79±0.25	25.18±0.21	23.13±0.18
Total n-3/total n-6	0.74±0.01	0.49±0.01	0.62±0.03	0.56±0.03	0.65±0.04	0.54±0.04	0.91±0.02	0.49±0.01	0.80±0.04
HH	2.87±0.01	3.70±0.09	3.02±0.13	3.46±0.03	2.71±0.09	3.60±0.14	2.82±0.01	3.63±0.02	3.01±0.04

[1]Each value represents the mean value ± standard deviation of three samples of the extracted fat per group, analysed twice (n=6). ND – not detected, detection limit 0.05%

Abbreviations: SFA, saturated fatty acids, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids,

HH, hypocholesterolaemic/hypercholesterolaemic ratio = (C18:1 n-9+ C18:2 n-6+C20:4 n-6 + C18:3 n-3+C20:5n-3+ C22:5 n-3+ C22:6n-3)/(C14:0+C16:0)

Table 5 - Fatty acid composition (%) of the sea bream[1].

	June			October			January		
Fatty acid	**Farm 1**	**Farm 2**	**Farm 4**	**Farm 1**	**Farm 2**	**Farm 4**	**Farm 1**	**Farm 2**	**Farm 4**
C14:0	3.67±0.13	4.29±0.16	3.09±0.10	5.41±0.30	2.75±0.11	2.95±0.30	2.71±0.34	3.32±0.44	2.77±0.45
C15:0	0.30±0.02	0.43±0.03	0.38±0.04	0.38±0.05	0.24±0.03	0.27±0.06	0.24±0.02	0.26±0.01	0.23±0.01
C16:0	15.87±0.31	17.99±0.26	15.37±0.44	20.77±0.11	13.77±0.24	14.55±0.51	14.09±0.42	15.25±0.24	15.00±0.42
C16:1n-7	4.61±0.16	5.35±0.27	4.59±0.33	6.19±0.16	4.41±0.17	4.43±0.22	3.65±0.24	4.85±0.23	3.99±0.16
C17:0	0.40±0.02	0.58±0.02	0.68±0.04	0.49±0.04	0.44±0.02	0.46±0.02	0.42±0.14	0.57±0.04	0.41±0.03
C17:1n-7	0.06±0.01	0.20±0.02	0.29±0.04	0.11±0.01	0.21±0.02	0.20±0.03	0.21±0.03	0.32±0.02	0.20±0.02
C18:0	3.92±0.27	4.55±0.29	3.46±0.21	3.94±0.17	3.28±0.19	3.40±0.12	3.67±0.13	4.07±0.54	3.29±0.27
C18:1n-9	25.74±0.37	27.11±0.34	26.51±0.27	30.68±0.30	28.02±0.34	30.02±0.91	27.79±0.32	25.27±0.46	28.44±0.73
C18:1n-7	2.50±0.04	2.67±0.15	2.42±0.12	3.15±0.12	2.32±0.05	2.46±0.17	2.01±0.20	2.97±0.57	2.44±0.53
C18:2n-6	22.96±0.38	22.61±0.28	24.93±0.27	10.92±0.18	24.20±0.32	22.46±0.44	27.32±0.46	25.19±0.62	25.80±0.19
C18:3n-6	0.22±0.01	0.05±0.01	0.26±0.01	0.09±0.01	0.21±0.02	0.23±0.08	0.23±0.01	0.21±0.05	0.30±0.02
C18:3n-3	2.90±0.15	2.09±0.15	3.41±0.17	1.43±0.15	4.51±0.25	5.21±0.54	5.37±0.10	3.01±0.07	5.34±0.07
C18:4n-3	0.76±0.10	0.46±0.01	0.60±0.04	0.45±0.02	0.57±0.03	0.59±0.02	0.52±0.11	0.63±0.11	0.51±0.03
C20:0	0.31±0.01	0.41±0.02	0.28±0.03	0.31±0.02	0.38±0.04	0.35±0.02	0.27±0.03	0.23±0.02	0.21±0.01
C20:1n-9	2.86±0.28	1.85±0.24	1.97±0.10	5.00±0.20	1.74±0.14	1.72±0.36	1.54±0.23	1.67±0.31	1.59±0.38
C20:2n-6	0.67±0.07	0.61±0.02	0.80±0.05	0.14±0.03	0.76±0.21	0.64±0.22	0.66±0.02	0.59±0.12	0.65±0.05
C20:3n-6	0.26±0.07	0.07±0.02	0.08±0.03	0.11±0.01	0.24±0.10	0.19±0.08	0.22±0.02	0.22±0.02	0.21±0.01
C20:4n-6	0.28±0.02	0.32±0.11	0.38±0.03	0.14±0.02	0.25±0.11	0.23±0.04	0.22±0.03	0.33±0.01	0.21±0.01
C20:3n-3	0.20±0.02	0.40±0.12	0.06±0.01	0.11±0.03	0.31±0.17	0.26±0.02	0.28±0.05	0.16±0.01	0.27±0.02
C20:4n-3	0.52±0.06	0.48±0.15	0.42±0.11	0.32±0.02	0.35±0.03	0.33±0.01	0.47±0.02	0.49±0.02	0.47±0.02
C22:0	ND	0.11±0.02	0.07±0.01	0.15±0.01	0.19±0.05	0.16±0.04	0.20±0.02	0.16±0.02	0.16±0.00
C20:5n-3	2.04±0.26	1.48±0.15	3.46±0.38	0.79±0.26	2.45±0.02	2.15±0.40	1.96±0.22	2.96±0.43	1.97±0.09
C22:1n-11	2.81±0.15	1.40±0.12	1.00±0.03	5.64±0.19	1.04±0.31	1.04±0.08	0.70±0.13	0.76±0.14	0.72±0.04
C22:1n-9	0.55±0.03	0.43±0.02	0.19±0.02	0.81±0.32	0.41±0.09	0.39±0.02	0.32±0.02	0.29±0.04	0.31±0.01
C22:5n-3	1.49±0.27	1.07±0.16	0.82±0.11	0.60±0.07	2.01±0.26	1.45±0.43	1.36±0.31	2.04±0.43	1.34±0.41
C24:1n-9	0.54±0.04	0.49±0.11	0.14±0.04	0.65±0.13	0.36±0.11	0.37±0.04	0.32±0.02	0.28±0.02	0.14±0.03
C22:6n-3	3.58±0.28	2.48±0.23	4.34±0.40	0.94±0.18	4.56±0.23	3.47±0.47	3.19±0.33	3.89±0.32	3.04±0.32
SFA	24.52±0.21	28.42±0.28	23.34±0.34	31.46±0.15	21.06±0.48	22.14±0.52	21.60±0.88	23.86±0.29	22.08±0.64
MUFA	39.61±0.29	39.51±0.47	37.11±0.24	52.33±0.29	38.50±0.47	40.64±1.04	36.52±0.34	36.40±0.38	37.76±0.46
PUFA	35.87±0.41	32.07±0.31	39.56±1.21	16.21±0.34	40.44±0.24	37.22±0.56	41.87±0.75	39.73±0.57	40.16±0.58
Total n-3	11.49±0.37	8.46±0.20	13.11±0.65	4.64±0.31	14.77±0.29	13.46±0.69	13.13±0.70	13.18±0.40	12.94±0.49
Total n-6	24.39±0.25	23.61±0.21	26.45±0.48	11.57±0.19	25.67±0.21	23.76±0.87	28.74±0.25	26.55±0.37	27.22±0.48
Totak n-3/total n-6	0.47±0.01	0.36±0.01	0.50±0.04	0.40±0.01	0.58±0.01	0.57±0.02	0.46±0.03	0.50±0.01	0.48±0.01
HH	3.08±0.03	2.63±0.08	3.54±0.09	1.84±0.03	4.00±0.08	3.77±0.08	4.04±0.02	3.42±0.02	3.78±0.02

[1]Each value represents the mean value ± standard deviation of three samples of the extracted fat per group, analysed twice (n=6). ND – not detected, detection limit 0.05%

Abbreviations: SFA, saturated fatty acids, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids,

HH, hypocholesterolaemic/hypercholesterolaemic ratio = (C18:1 n-9+ C18:2 n-6+C20:4 n-6 + C18:3 n-3+C20:5n-3+ C22:5 n-3+ C22:6n-3)/(C14:0+C16:0)

confirmed an increase in fatty acids with 18C such as OA, LA and ALA in farmed fish, gained through the use of vegetable oils in their feed. The concentration of long- chain polyunsaturated acids – eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) - in total fatty acids was in range of 2.18-6.65% for EPA and 3.21-6.58% for DHA in sea bass samples, and in the range of 0.79-1.48% for EPA and 0.94-3-19% for DHA in sea bream samples. These results are due to the feeding strategy, since it is well known that fatty acid composition in fish meat reflects dietary fatty acid profile. Over the last decades, fish nutrition research has devoted continued effort to the development of sustainable feeds that can provide long-chain n-3 fatty acid levels adequate for human nutrition (IZQUIERDO *et al.*, 2005; KRIS-ETHERTON *et al.*, 2003).

It has been suggested that "n-3/n-6 ratio" represents a reliable index for inter-species comparisons of relative nutritional values (PIGGOT and TUCKER, 1990). According to SARGENT (1997), the optimum n-3/n-6 PUFA ratio should be 1:5 (0.2). Generally fish have this ratio more favourable as also evident from the results shown in Tables 4 and 5. However, our results showed lower n-3/n-6 ratios in comparison to comparative studies of farmed and wild sea bass and sea bream (ALASALVAR *et al.*, 2002; FUENTES *et al.*, 2010; PERIAGO *et al.*, 2005; SAGLIK *et al.*, 2003). ORBAN *et al.* (2003) studied lipid quality of wild fish and fish farmed in North Adriatic, and also obtained n-3/n-6 ratio higher than ours. Similar or even lower n-3/n-6 ratios were presented by CARDINAL *et al.* (2011), who studied seasonal variations of physico-chemical and sensory characteristics of sea bream coming from the market. These differences in n-3/n-6

ratios are caused by the differences in formulation of diets used in fish farms. It is obvious that farms that were compared to the wild fish have better adapted their fish meals. However, the recent research conducted by SOFI and co-workers (2013) confirmed that the intake of fish with similar EPA+DHA content but different n-3/n-6 ratio has different effects on lipid, inflammatory and haemoreological parameters of healthy subjects.

As it is formally recommended to humans to take 0.3 to 0.5 g of n-3 fatty acids (EPA + DHA) per day (PRATOOMYOTET *et al.*, 2010), consumers´ weekly needs should be satisfied with the consumption of approximately 600 g of sea bass or sea bream. Consumers with coronary heart disease should be encouraged to increase their daily consumption to even 200 g of sea bass or sea bream.

Due to the known effects of specific fatty acids on cholesterol metabolism, one of the indicators of nutritional quality may also be the ratio between hypocholesterolaemic and hypercholesterolaemic fatty acids (HH) (SANTOS-SILVA *et al.*, 2002; TESTI *et al.*, 2006). Generally, sea bream had higher HH index due to the lowest share of saturated fatty acids. The highest HH value, i.e. the most desirable one (4.04), was found in the sea bream sampled in January.

The influence of season and location on fatty acid composition of both fish species is presented in Table 6. Statistical analysis showed the influence of these parameters on the sea bass to be stronger than on the sea bream. As for the sea bass, significant difference ($p<0.05$) in the content of almost all n-3 fatty acids was shown, resulting in differences in both total n-3 fatty acid content and n-3/n-6 ratio. Although diet is the main factor that affects n-3 and n-6 PUFA content in fish, location, species, season and environmental conditions may also play a role (HOSSAIN, 2011). CORDIER *et al.* (2002) also reported differences in n-3/n-6 ratio, especially the difference in EPA over AA ratio. That is in accordance with our results, although within the frame of our study arachidonic acid ($p=0.012$) was influenced by the season, and not by the farming location. EPA over AA ratio is considered to be an important parameter, since dietary intake of n-3 PUFAs helps replacing, at least to a point, n-6 fatty acids in cell membranes, most importantly in platelet, erythrocyte and neutrophil cell membranes (SIMOPOULOS, 2002). CARDINAL *et al.* (2011) also reported significant season-dependent differences in fatty acid content, but this was attributed to the rearing conditions, feed formulation included, which were actually not controlled within their study. Significant variations in some fatty acids present in fish meat, especially variations in linoleic acid (C18:2 n-6) ($p=0.013$), possibly arise as a consequence of different plant contents present in the diets selected as common feed by various farms.

Muscle protein content may be less important than the fat one, but proteins, especially those interacting with water, contribute to organoleptic quality of the fish meat (ZAYAS, 1997). From the nutritional point of view, fish proteins are important since, according to the recent FAO data, fish accounts for 15.7% of the global population's animal protein intake and 6.1% of the entire protein intake (FAO, 2012). In this study, crude protein content varied in range from 18.89% to 22.52% for sea bass, and 18.76% to 21.73% for sea bream, in accordance with the proximate values obtained in earlier studies, summarized in the review of GRIGORAKIS (2007).

Depending on the species, collagen and hydroxyproline fish meat contents vary in range from 0.28% to 0.79% and 30 to 98 mg/100 g, respectively (MORRISEY and FOX, 1981). Values

Table 6 - Statistical analyses of fatty acid profile (ANOVA) of the sea bass and sea bream.

	Sea bass (p values)		Sea bream (p values)	
Fatty acid	**Season**	**Location**	**Season**	**Location**
C14:0	0.715	0.184	0.521	0.315
C15:0	0.556	0.392	0.216	0.947
C16:0	0.258	0.463	0.672	0.473
C16:1n-7	0.841	0.476	0.344	0.278
C17:0	0.244	0.719	0.408	0.859
C17:1n-7	0.909	0.597	0.665	0.523
C18:0	0.225	0.196	0.538	0.514
C18:1n-9	0.069	0.497	0.132	0.778
C18:1n-7	0.565	0.238	0.795	0.171
C18:2n-6	0.210	0.013*	0.200	0.310
C18:3n-6	0.183	0.220	0.610	0.683
C18:3n-3	0.714	0.528	0.262	0.151
C18:4n-3	0.043*	0.106	0.764	0.596
C20:0	0.089	0.419	0.083	0.477
C20:1n-9	0.052	0.001*	0.380	0.216
C20:2n-6	0.406	0.859	0.538	0.333
C20:3n-6	0.417	0.095	0.650	0.932
C20:4n-6	0.012*	0.153	0.242	0.913
C20:3n-3	0.004*	0.015*	0.722	0.133
C20:4n-3	0.053	0.407	0.001	0.563
C22:0	0.243	0.433	0.027	0.255
C20:5n-3	0.120	0.328	0.762	0.717
C22:1n-11	0.153	0.002*	0.329	0.200
C22:1n-9	0.983	0.090	0.257	0.209
C22:5n-3	0.009*	0.009*	0.681	0.868
C24:1n-9	0.117	0.112	0.311	0.313
C22:6n-3	0.041*	0.310	0.892	0.800
SFA	0.301	0.624	0.627	0.437
MUFA	0.153	0.915	0.246	0.431
PUFA	0.066	0.135	0.420	0.419
Total n-3	0.033*	0.193	0.736	0.549
Total n-6	0.326	0.014*	0.415	0.560
Total n-3/total n-6	0.038*	0.092	0.442	0.439

*significantly different ($p<0.05$).

obtained in this study for both fish types are within this range. Other studies reported lower collagen values in farmed fish in comparison to the wild one, which was presumed to be related to the swimming behaviour (SATO *et al.*, 1986), as well as to other factors such as a higher number of muscle fibres which predestines for greater collagen content (SIKORSKI *et al.*, 1984).

The main functions of fish minerals include skeleton structuring, maintenance of colloidal system and regulation of acid-base equilibrium; in addition, these minerals also represent the important hormone, enzyme and enzyme activator constituents (BELITZ and GROSCH, 2001). Literature data have demonstrated that the origin of fish and their feeding pattern did not have any effect on mineral composition, except for that on calcium content (FUENTES *et al.*, 2010).

Calcium (Ca) and phosphorus (P) are necessary for maintaining optimal bones development, being a higher intake of both minerals required during childhood and growing ages so as to prevent rickets and osteomalacia (ERKAN and ÖZDEN, 2007). The content of these minerals determined in sea bass of this study, ranged from 662 to 813 mg/kg and from 3589 to 3913 mg/kg, respectively, while that in sea bream from 241 to 281 mg/kg and from 3321 to 3551 mg/kg, respectively. As reported earlier by ERKAN and ÖZDEN (2007), mean contents of these minerals in sea bass were also significantly higher ($p<0.05$) than those determined in sea bream. Furthermore, obtained results for Ca content in sea bream are in a good agreement with results published by ORBAN *et al.* (2003) whose results ranged from 220 to 230 mg/kg.

As for Ca, sodium (Na) content found in sea bass was significantly higher than in sea bream. Results obtained for sea bass ranged from 515 to 738 mg/kg, while for sea bream ranged from 268 to 365 mg/kg. Literature have reported sea bream Na contents ranging from 280 to 370 mg/kg and sea bass Na contents of 773±1.8 mg/kg (ORBAN *et al.*, 2003). Other sources reported Na content about 289±1.6 mg/kg in sea bream (ERKAN and ÖZDEN, 2007), which is quite similar to the data obtained within this study.

CONCLUSION

Results of our study clearly show seasonal variations of moisture and fat content in both fish species. As for sea bass, differences in fatty acid composition were shown, while in sea bream these differences were not observed. The n-3/n-6 ratios were lower than those previously reported for farmed and wild fish. Fish nutritional value is related to n-3 fatty acids, and is heavily dependent on the production process. Since fish is promoted as a good source of n-3 fatty acids, efforts should be made to properly tailor lipid quality, basically relying on dietary manipulation so as to fit fat deposition and fatty acid profile. Seasonal and location changes did not affect mineral composition, but mutual difference between the two species under study was significant.

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