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Evaluation of the fillet quality of wild-caught white sea bream (*Diplodus sargus* L.) and brown meagre (*Sciaena umbra* L.) captured from the Aegean Sea

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Abstract

Wild white sea bream (*Diplodus sargus*) and brown meagre (*Sciaena umbra*) were caught from the Aegean Sea (Greece), and studied for their proximate, fatty acid and amino acid composition to evaluate their nutritional value for human consumption and their potential as candidate fish species for Mediterranean aquaculture diversification. Both species exhibited very low muscle fat, ranging at 1%. White sea bream was found to have higher muscle eicosapentaenoic (EPA) and docohexaenoic (DHA) contents and total n-3 fatty acids than brown meagre. A superiority of white sea bream with respect to thrombogenicity was found, with higher atherogenic (0.679) and thrombogenic indices (0.377) compared to the respective values for brown meagre (0.610 and 0.579). Both studied species exhibited high-quality protein, with a higher essential to non-essential amino acid (EAA/NEAA) rate for white sea bream, 0.764 vs 0.704, respectively. The individual to total essential amino acids rates of lysine and leucine were the highest ones for both studied species, while no significant differences were observed among them.

Keywords: Nutritional quality; *Diplodus sargus*; *Sciaena umbra*; composition; essential amino acids; essential fatty acids.

Introduction

During the last decade, the oversupply of gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) had resulted in market saturation; prices are declining and there are signs of a crisis in Mediterranean aquaculture (Barazi-Yeroulanos, 2010; Oztekin *et al.*, 2018), thus indicating the need for diversification. Therefore, the introduction of new marine species may increase product diversity and create new market opportunities, and is considered as a basic alternative for overcoming this problem in Mediterranean aquaculture (Cardia & Lovatelli, 2007; Sahinyılmaz & Yigit, 2017). Among the candidate species, white sea bream (*Diplodus sargus*) and brown meagre (*Sciaena umbra*) are considered as potential candidates for diversification of Mediterranean aquaculture. Both species have been successfully reared on a small scale, with successful reproduction and on-growing (Barazi-Yeroulanos, 2010).

One of the basic aspects in establishing the production of new species is to identify the quality characteristics of wild individuals of the same species. In this way, useful information is gained with respect to the nutritional needs

of the species and the desired quality of the product (Kaushik, 1998).

The first data regarding the fillet quality of white sea bream and brown meagre under farming conditions have been reported by Cejas *et al.* (2004) and Cakli *et al.* (2006).

Some data are available regarding the composition (Saoud *et al.*, 2008) and fatty acids (Hornung *et al.*, 1994; Cejas *et al.*, 2004; Özyurt *et al.*, 2005; Pérez *et al.*, 2007) of wild-caught white sea bream and some, only sporadic, regarding the composition and fatty acids of wild-caught brown meagre (Cakli *et al.*, 2006). However, as far as we know, no field data about the fillet amino acid composition of these two species is available. Indeed, the amino acid and fatty acid composition provide useful information on the nutritional value of these two fish species, which determine fish quality as perceived by the consumer (Grigorakis, 2007). Furthermore, information on the fillet amino acids of these species would be useful for gaining knowledge on their amino acid dietary requirements (Kaushik, 1998). Thus, the aim of this study was to compare the fillet quality of wild-caught white sea bream and brown meagre captured from the Aegean Sea, as regards proximate composition, fatty acids and amino acid contents.

Materials and Methods

Specimens of both species were collected by harpoon-fishing at depths between 5 and 15 meters, in the NE of the island of Lesbos, Greece (Aegean Sea). The sampling area is located between Mithymna (39.36791° N, 26.17580° E) and Cape Korakas (39.38549° N, 26.34012° E). Amateur marine fishing is permitted only in April and June but not in May, because of the fishing ban period imposed on sport-recreational fishing by Presidential Decree No. 373 dated 16 July 1985. The respective average water temperature range is from 15.5 to 18.5 °C.

Fish were placed on ice immediately after fishing, manually filleted and placed in a freezer at -30°C upon arrival at the Laboratory of Fish Nutrition (HCMR, Athens).

In total, 10 individuals of white sea bream (21.4 ± 1.35 cm length, 210.5 ± 6.82 g total body weight) and 10 individuals of brown meagre (25.0 ± 1.74 cm length, 200.8 ± 5.00 g total body weight) were individually analyzed for all the studied parameters.

The fish fillets were homogenized and proximate composition was analyzed for dry matter, ash, crude protein and crude fat, according to AOAC (1998). Moisture content was measured after drying the samples at 105°C for 24 h; ash was determined after ignition at 500°C for 12 h; crude protein content was analyzed using the Kjeldahl method (N × 6.25) (Kjeltec 8100, FOSS, Denmark) and crude fat was estimated gravimetrically in freeze-dry muscle samples by Soxhlet extraction using Soxtec™ apparatus (FOSS, 2050 automated analyzer, Denmark) and petroleum ether as solvent. Gross energy of the diets was determined by an adiabatic bomb calorimeter (IKA, Werke GmbH).

Fatty acid methyl esters (FAMES) in muscle were determined by a modified direct transesterification method proposed by Lepage & Roy (1984). Muscle samples (300 - 400 mg) from each species were weighed in tubes to which 5 mL of methanol/toluene (3/2 v/v) and 5 mL of freshly prepared acetylchloride/methanol (1/20 v/v) were added. The tubes containing this mixture were incubated at 100°C for 60 min, and allowed to cool at room temperature. Then, 5 mL of water and 5 mL of hexane were added, the tubes were shaken, centrifuged and the hexane layer was taken for the FAME analyses. The FAME analyses were then analyzed using an Agilent GC-7890 B gas chromatograph (Agilent Technologies, Santa Clara, CA, USA), equipped with a flame-ionization detector (GC-FID) and a DB-23 capillary column (60 m x 0.25 mm i.d. × 0.15 µm film thickness) (Agilent, Santa Clara, CA, USA). Helium was used as carrier gas at 2 mL/min constant flow; the split ratio was 1:50 and the injected volume 1.0 µL. The thermal gradient was 50°C for 1 min, 50°C to 175°C at 25°C/min, 175°C to 230°C at 4°C/min and kept at 230°C for 15 minutes. The injector and detector temperature were maintained at 250 and 280°C, respectively. Fatty acids were identified by comparison with a known standard mixture (Supelco 37 Component FAME Mix). Fatty acid methyl ester contents in feed and muscle tissue were expressed as a % of total FAMES basis.

Regarding the nutritional value of fillet lipids, the related health lipid indices such as the atherogenic index (AI) and the thrombogenic index (TI) were also calculated for both species. For this purpose, the Ulbricht & Southgate (1991) formulas were used: $AI = [12:0 + (4 \times 14:0) + 16:0] / (\text{Sum MUFA} + \text{Sum PUFA})$ and $TI = (14:0 + 16:0 + 18:0) / [0.5 \times \text{Sum MUFA} + 0.5 \times \text{Sum (n-6) PUFA} + 3 \times \text{Sum (n-3) PUFA} + (n-3 / n-6)]$, where MUFA are the monounsaturated fatty acids and PUFA the polyunsaturated fatty acids.

The amino acid composition of the diets was analyzed after acid hydrolysis (6 N HCl, 110°C, 24 h) and derivatization by AccQ-Tag™ Ultra according to the amino acid analysis application solution (Waters Corporation, Milford, MA, U.S.A.). DL- Norvaline (Sigma) 2.5 mM was used as an internal standard. UPLC was performed on an Acquity system (Waters Corporation, Milford, MA, U.S.A.) equipped with a PDA detector and the detection wavelength was set at 260 nm. The column used was a Waters BEH C₁₈ column (100 mm × 2.1 mm i.d., 1.7 µm). The flow rate was 0.7 mL/min and the column temperature was kept at 55°C. Peak identification and integration was performed using Empower v.2.0 software (Waters Corporation, Milford, MA, U.S.A.) and Amino Acid Standard H (Thermo Scientific Pierce) as an external standard. All analyses were performed in duplicate. In cases where the values between replicates didn't meet the standardized acceptance criteria based on the standard deviation of the mean (< 5%), new duplicate analyses were performed according to established procedures. Tryptophan was not quantified due to its susceptibility to acid hydrolysis, while cystein reacts with cysteine to form cystine. Moreover, during the acid hydrolysis procedure, asparagine is converted to aspartate and glutamine to glutamate, so the reported values for these amino acids (Asx and Glx) represent the sum of both amino acids.

For the evaluation of fish protein quality, the A/E (the ratios of the contents of individual essential amino acids to the total essential amino acids content) and EAA/NEAA ratios (molar ratio of essential to non-essential amino acids) were also calculated (Swendseid *et al.*, 1963; ElShehawy *et al.*, 2016).

Statistical differences in the studied parameters of the two fish species were evaluated by Student's t-test (P = 0.05) using SPSS 13.0 software.

Results and Discussion

Although sex and maturity of fish have not been evaluated, maturity can be speculated by the capture period. In the Mediterranean basin, brown meagre spawns between May and August (Grau *et al.*, 2009), while the spawning season of white sea bream is in early-middle spring (Mouine *et al.*, 2012). Thus, it is assumed that the current sampling coincides with the sexual maturity period of brown meagre, but not that of white sea bream.

The fillet proximate composition of the two species is presented in Table 1. Both species have a total lipid content of around 1%.

The protein content of white sea bream was similar to that mentioned in the literature for wild individuals (Özyurt *et al.*, 2005; Saoud *et al.*, 2008). However, the protein content of brown meagre found in the current study was lower than the percentage (23%) reported by Cakli *et al.* (2006).

The lipid content of white sea bream was found to be lower than 2.37%, as reported by Özyurt *et al.* (2005), but within the range of 0.35 - 1.85% found by Saoud *et al.* (2008) for wild individuals, for the same period of the year. It is also lower than the values mentioned for the same fish species during the middle and end of the summer (Hornung *et al.*, 1994; Özyurt *et al.*, 2005; Saoud *et al.*, 2008). The lipid content of brown meagre is slightly lower than that mentioned in the only available study on wild specimens of the same species carried out by Cakli *et al.* (2006), who found a content of 1.48% for individuals caught in the Aegean Sea during the same period of the year.

In general, members of the sciaenidae family, to which brown meagre belongs, tend to accumulate very low muscle fat, irrespective of their dietary history and their origin, fisheries or aquaculture (Poli *et al.*, 2003; Özyurt *et al.*, 2005; Segato *et al.*, 2005a; Segato *et al.*, 2005b; Cakli *et al.*, 2006; Grigorakis *et al.*, 2011; Grigorakis, 2017).

Although, being a Sciaenidae, brown meagre is considered a low-fat species while white sea bream, like Mediterranean Sparidae, is a medium-fat species (Grigorakis, 2017), in this study they were both found to have similar fat levels (Table 1). The fact that the former is near its fat accumulation peak when approaching the spawning season, while the latter is at the end of the spawning period, prior to the abundant summer feeding, provides a possible explanation for their fillet fat content.

The fatty acid composition of the two studied species is presented in Table 2. White sea bream was found to have higher total n-3 contents, almost 1.5 times higher 20:5n-3 (eicosapentaenoic acid, EPA) and 2.5 times higher 22:6n-3 (docohexaenoic acid, DHA) than brown meagre. These differences between the two species are due to a different lipid metabolism as well as different dietary habits since tissue fatty acids reflect the dietary fatty acids (Corraze, 2001).

The major fatty acid groups in white sea bream were found to differ from those mentioned by Cejas *et al.* (2004) who found lower saturates and monounsaturates

but higher n-3 and n-6 polyunsaturates, although quite similar to those reported by Özyurt *et al.* (2005). With respect to individual fatty acids, white sea bream was found to have lower arachidonic acid (AA) and fairly similar EPA contents compared to those mentioned in the literature (Hornung *et al.*, 1994; Cejas *et al.*, 2004; Özyurt *et al.*, 2005). As regard DHA, it was found to be lower than that mentioned by Cejas *et al.* (2004), similar to that of Özyurt *et al.* (2005), and higher than that reported by Hornung *et al.* (1994).

For wild brown meagre, data referring to individual fatty acids is scarce, but in the present research the values of fatty acid classes were found to differ from the values reported by Cakli *et al.* (2006), who mentioned higher saturated fatty acids (50.5%) and lower n-3 (9.84%) and n-6 (2.77%) for wild fish caught in the Mediterranean Sea in May. However, the highly unsaturated n-3 fatty acids (mainly EPA and DHA) in the aforementioned study were quite similar (8.44%) to our present results.

These differentiations in the total fat and fatty acids profile of wild individuals of the same species, could be explained by the trophic diversification associated with habitat transitions (Grigorakis, 2007; Benchalel *et al.*, 2010), genetic issues (species-specific factor) but also by the maturation and spawning process. This hypothesis has a strong basis if one considers that the spawning season of brown meagre peaks in May and June in the Mediterranean Sea (Grau *et al.*, 2009), while for white sea bream spawning takes place in March - April (Benchalel & Kara, 2013). Thus, our samples were probably collected during the spawning period in the case of brown meagre and after spawning in the case of white sea bream.

Individual fatty acids have been found to differ significantly in white sea bream, even when caught in the same geographical area in the Mediterranean Sea, but at different sites (Hornung *et al.*, 1994). Furthermore, distinctive changes in fatty acids have been observed during maturation and spawning (Pérez *et al.*, 2007).

It is well-known that fish lipids, due to their high n-3 PUFAs content, have a beneficial effect on human health by reducing the levels of cholesterol, triglycerides and the level of low-density lipoprotein cholesterol in blood serum, thus reducing the risk of atherosclerosis, stroke and cardiovascular disease (Horrocks & Yeo, 1999; Li *et al.*, 2003). The atherogenic and thrombogenic indices indicate the relations between saturated (pro-atherogenic) and unsaturated fatty acids (anti-atherogenic), which

Table 1. Fillet proximate composition (%) of white sea bream (*Diplodus sargus*) and brown meagre (*Sciaena umbra*).

| Proximate composition | White sea bream | Brown meagre |
|-----------------------|-----------------|--------------|
| Protein | 19.1 ± 0.84 | 18.7 ± 0.75 |
| Fat | 1.09 ± 0.09 | 1.08 ± 0.08 |
| Moisture | 78.2 ± 0.80 | 79.2 ± 0.72 |
| Ash | 1.46 ± 0.16 | 1.20 ± 0.04 |
| Energy (MJ/kg) | 4.98 ± 0.12 | 4.83 ± 0.10 |

Data are means, n=10 ± SD. Row means that share an asterisk are significantly different from each other (t-test, p < 0.05)

Table 2. Fatty acid composition (expressed as weight percent of total fatty acids) of white sea bream and brown meagre fillet.

| Fatty acids | White sea bream | Brown meagre | P-value |
|-------------------------|-----------------|--------------|---------|
| 14:0 | 5.07 ± 0.39 | 2.94 ± 0.16 | * |
| 14:1 | 0.36 ± 0.07 | 0.34 ± 0.10 | |
| 15:0 | 1.11 ± 0.52 | 1.23 ± 0.47 | |
| 15:1 | 0.25 ± 0.33 | 0.43 ± 0.39 | |
| 16:0 | 21.9 ± 1.50 | 24.3 ± 0.58 | * |
| 16:1n-9trans | 1.54 ± 0.25 | 0.51 ± 0.12 | ** |
| 16:1n-9cis | 7.09 ± 1.30 | 5.80 ± 0.59 | * |
| 16:2n-4 | ND | ND | |
| 17:0 | 1.04 ± 0.54 | 1.23 ± 0.98 | |
| 16:3n-3 | ND | ND | |
| 16:3n-4 | ND | ND | |
| 17:1n-9 | 0.73 ± 0.23 | 1.1 ± 0.31 | |
| 16:4n-3 | ND | ND | |
| 18:0 | 7.18 ± 0.12 | 10.08 ± 0.15 | * |
| 18:1n-9trans | 0.47 ± 0.09 | 0.43 ± 0.12 | |
| 18:1n-9cis | 17.0 ± 0.21 | 23.4 ± 0.33 | * |
| 18:1n-7 | 4.02 ± 0.21 | 4.58 ± 0.43 | |
| 18:2n-6trans | 0.41 ± 0.62 | 0.87 ± 0.51 | |
| 18:2n-6cis | 3.24 ± 1.51 | 2.83 ± 0.60 | |
| 18:3n-3trans | 1.16 ± 0.11 | 0.96 ± 0.04 | |
| 18:3n-3cis | 1.58 ± 0.39 | 1.39 ± 0.52 | |
| 20:0 | 0.54 ± 0.25 | 0.70 ± 0.32 | |
| 20:1n-9 | 2.18 ± 0.11 | 1.78 ± 0.49 | |
| 20:4n-6 (AA) | 2.20 ± 0.21 | 2.50 ± 0.31 | |
| 20:5n-3 (EPA) | 8.15 ± 0.31 | 5.33 ± 0.59 | ** |
| 22:0 | 0.93 ± 0.40 | 0.54 ± 0.47 | |
| 22:1n-9 | 0.37 ± 0.09 | 1.30 ± 0.31 | * |
| 22:5n-3 | 3.69 ± 0.37 | 2.23 ± 0.72 | * |
| 24:0 | ND | ND | |
| 22:6n-3 (DHA) | 7.76 ± 0.52 | 3.24 ± 0.31 | ** |
| 24:1n-9 | ND | ND | |
| Saturates | 37.77 | 41.02 | |
| MUFA¹ | 34.01 | 39.67 | |
| PUFA² | 28.19 | 19.35 | |
| n-9 | 29.38 | 34.32 | * |
| PUFA n-6 | 5.85 | 6.20 | |
| PUFA n-3 | 22.34 | 13.15 | * |
| EPA + DHA | 15.91 | 8.57 | |
| n-3 / n-6 | 3.82 | 2.12 | * |
| AI³ | 0.678 | 0.611 | |
| TI⁴ | 0.376 | 0.579 | * |

ND = Not Detected

Data are means, n=10 ± SD. Row means that share an asterisk are significantly different from each other (t-test, *p < 0.05, **p < 0.01). ¹- Monounsaturated fatty acids. ²- Polyunsaturated fatty acids. ³- Atherogenic Index. ⁴-Thrombogenic Index.

have been linked with the diet-heart hypothesis and are related with the incidence of coronary disease, thrombus and atheroma formation (Ulbricht & Southgate, 1991; Zock *et al.*, 2016).

As regard the nutritional value of the lipids, the atherogenic index of white sea bream was found to be 0.678 and the thrombogenic index was 0.376, while the respective values for brown meagre were 0.611 and 0.579. The TI values indicate superiority for white sea bream as regards the quality of its lipids.

Comparing these values for white sea bream with those of other species of Sparidae, we observed a lower AI and a higher TI than the values found (1.18 and 0.29, respectively) for the bogue *Boops boops* (Kalogeropoulou *et al.*, 2004). The AI found in this study was higher and the TI was similar to that of wild-caught gilthead sea bream (*Sparus aurata*) (Grigorakis, 2007).

The fillet amino acid contents are presented in Table 3. Glutamic acid, aspartic acid and lysine are the most abundant, sorted by decreasing concentrations, followed by leucine, agrinine and alanine. Comparing the present data with the respective values found for wild European sea bass (Özyurt & Polat, 2006; Erdem *et al.*, 2009) we note that Lys is the most abundant amino acid in sea bass, followed by Asp, Glu and Leu (Özyurt & Polat, 2006; Erdem *et al.*, 2009). The most important difference in amino acid patterns is related to the Arg content, which is lower in wild sea bass (Özyurt & Polat, 2006; Erdem *et al.*, 2009).

The EAA/NEAA (> 0.70) ratios indicate that both species can be considered as high-quality sources of protein (Pinto *et al.*, 2007). However, the protein nutritional value of the two studied species differs significantly, with white sea bream exhibiting superiority compared to brown meagre, with a higher EAA/NEAA rate for white sea bream, 0.764 vs 0.704 for brown meagre (Table 3). Regarding the individual to total essential amino acids rates (Fig. 1.), Lys and Leu were the most pronounced ones for both species, while no significant differences were observed between them. In general, the rates are quite similar to those reported for wild sea bass (Grigorakis, 2007). A small difference was observed for Leu, a higher rate was found for brown meagre, and for Val,

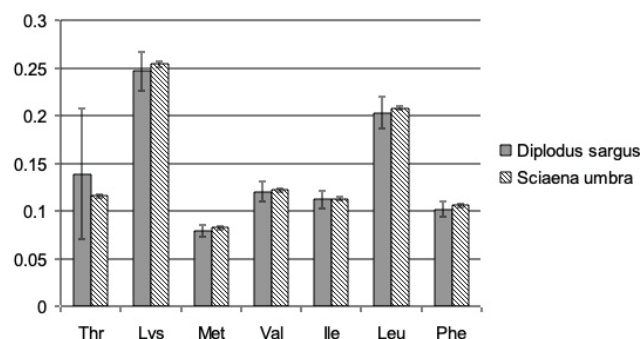


Fig. 1: A/E ratios for wild white sea bream and brown meagre. Data are means, n = 10 ± SD.

Table 3. Amino acid profile of white sea bream (*Diplodus sargus*) and brown meagre (*Sciaena umbra*) muscle (in g/100 g tissue) and essential (EAA) to non-essential (NEAA) amino acid ratio.

| Amino acids | White sea bream | Brown meagre | P-value |
|-------------------------|-----------------|---------------|---------|
| His | 0.351 ± 0.161 | 0.299 ± 0.107 | * |
| Thr | 1.269 ± 0.861 | 0.950 ± 0.063 | * |
| Lys | 2.147 ± 0.128 | 2.083 ± 0.116 | |
| Met | 0.686 ± 0.055 | 0.677 ± 0.044 | |
| Val | 1.047 ± 0.081 | 0.998 ± 0.062 | |
| Ile | 0.974 ± 0.063 | 0.923 ± 0.063 | |
| Leu | 1.768 ± 0.110 | 1.704 ± 0.096 | |
| Phe | 0.889 ± 0.072 | 0.870 ± 0.056 | |
| EAA^a | 9.131 | 8.504 | |
| Tau | 0.303 ± 0.040 | 0.493 ± 0.102 | ** |
| Ser | 0.786 ± 0.221 | 0.822 ± 0.074 | |
| Arg | 1.270 ± 0.175 | 1.271 ± 0.095 | |
| Gly | 1.007 ± 0.166 | 0.891 ± 0.105 | |
| Asx | 2.293 ± 0.495 | 2.366 ± 0.127 | |
| Glx | 3.455 ± 0.432 | 3.540 ± 0.188 | |
| Ala | 1.305 ± 0.151 | 1.297 ± 0.072 | |
| Pro | 0.767 ± 0.233 | 0.679 ± 0.049 | |
| Tyr | 0.762 ± 0.043 | 0.717 ± 0.087 | |
| NEAA^b | 11.948 | 12.076 | |
| EAA/NEAA | 0.764 | 0.704 | * |

^a – Essential amino acids. ^b – Non-essential amino acids.

Data are means, n = 10 ± SD. Row means that share an asterisk are significantly different from each other (t-test, *p < 0.05)

which is lower than the 0.15 reported for wild sea bass (Grigorakis, 2007).

Conclusively, the fatty acid and amino acid quality of both species is similar to the one characterizing most wild Mediterranean fish species (Grigorakis, 2017). Therefore, they can be considered as important source of nutrients for humans and candidate fish species for Mediterranean aquaculture diversification. All differences found between the two species, for both the fatty acid and amino acid composition, can be attributed to their biological differences, but also to genetic issues (species-specific factor), since they were collected from exactly the same region in the Aegean Sea; the time-period of collection, i.e. the environmental factor was less influential.

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