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Effect of Spawning on Furan Fatty Acid Profile of Edible Muscle and Organ Tissues from Sardine (*Sardina pilchardus*) and Anchovy (*Engraulis encrasicolus*)

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ABSTRACT: The total fatty acid compositions, especially the furan fatty acid (F-acids) profile, from edible muscle (fillet) and organ tissues (brain, eye, ovaries, and testes) of spawning and nonspawning sardine and anchovy were examined. The spawning season had no effect on F-acid profiles of the fillet and all organ tissues, for both fishes. However, differences among the F-acid fraction of the organ tissues were revealed. The F-acid portion was less than 1% of total fatty acid in all samples. Five F-acid congeners were detected in the fillet, eye, and gonads, while the brain contained only four F-acids. Unlike the F-acids, spawning season affected the docosahexaenoic acid (DHA) abundance in fillet and gonads. DHA enrichment occurred in fillets and gonads from spawning sardine and anchovy. The ratio ω3 PUFA/ω6 PUFA decreases between spawning and nonspawning fillets, thus the fillets from spawning fish have higher nutritional value.

KEYWORDS: *Engraulis encrasicolus, Sardina pilchardus, Adriatic Sea fish, spawning effect, fish organ tissues, edible muscle, furan fatty acids*

INTRODUCTION

Sardine (*Sardina pilchardus*, Walbaum, 1972) and anchovy (*Engraulis encrasicolus*, Linneus, 1758) are the most important commercial coastal pelagic species of the Mediterranean Sea, especially of the Adriatic Sea, where they represent 41% of total marine catches.[1−4] The edible muscle (fillet) of these fish is highly recommendable for human nutrition, as their short life cycle prevents the accumulation of dangerous substances (i.e., heavy metals, pesticides) in the tissues.[5−7] Furthermore, the edible muscle of anchovy and sardine contains more water than warm-blooded animals, shows low cholesterol values and richness in essential ω3 polyunsaturated fatty acids (ω3 PUFA) in its lipid fraction composition, and is more digestible.[8−12] It is well known that ω3 PUFA, such as eicosapentaenoic (C20:5 ω3, EPA), docosapentaenoic (C22:5 ω3, DPA), and docosahexaenoic (C22:6 ω3, DHA) acids, exert a healthy effect in coronary heart disease, the major cause of death in most developed countries. ω3 PUFA can be used as cancer chemopreventive agents and for the treatment of inflammatory lesions.[13−18] Recent studies have recognized that the positive effects of a diet rich in fish or fish oils on certain chronic diseases can be related to the presence of both ω3 PUFA and other bioactive components, such as furan fatty acids (F-acids). Even though F-acid concentration is usually low, these fatty acids are widely distributed in plants and aquatic organisms.[19−24] Okada et al. (1990) reported that F-acids exhibit potent radical-scavenging activity due to the electron-rich furan ring.[25] Spiteller (2005, 2007) hypothesized that they play a crucial nutritional role that is synergistic to ω3 PUFA, acting as efficient radical scavengers during PUFA oxidation.[26,27] Pacetti et al. (2010) demonstrated a good positive correlation between total F-acids and EPA contents in the fillet of six fish species from the Adriatic Sea, showing that the synthesis of F-acids with a skeletal formula of 20 carbon atoms is strictly related to EPA and is competitive with the elongation of EPA to DPA and its desaturation to DHA.[24] Wakimoto et al. (2011) demonstrated that F-acids of the lipid extract of the New Zealand green-lipped mussel exhibit more potent anti-inflammatory activity than that of EPA.[28]

However, difficulties arise when attempting to generalize about the fatty acid composition of fish species because of their great variability throughout the year due to both exogenous and endogenous factors, such as diet, size, age, reproductive cycle, temperature, season, and geographical location.[29−34] Experimental evidence suggests that the most important change in total lipid and fatty acid composition of fish is observed during the period of reproduction (spawning period). In this period, the storage lipids as well as other nutritional compounds (such as proteins, vitamins, and minerals) in muscle, liver, and visceral organs are mobilized to the gonads in order to ensure maturation.[35] Therefore, the nutritional quality of muscle may decrease during gonadal maturation.[36] According to Garrido, Rosa, Ben Hamadou, Cunha, Chicaro, and Van der Lingen (2008), the proportions of fatty acid fractions of Iberian sardine fillet remained fairly constant during most of the year but changed dramatically at the beginning of the spawning season, when the saturated fatty acid (SAT) amount increased substantially while the PUFA proportion decreased.[37] Tufan, Koral, and Kose (2011) indicated that although autumn

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represents higher fat content compared to other studied seasons for the edible parts of anchovies caught in the Black Sea, this species is more valuable during winter−spring in terms of total amount of ω3 PUFA. Indeed, DHA levels are higher in March−April than September, which represent the beginning and the end of the reproductive period, respectively.[38]

In particular, during the reproductive period there is a high requirement for ω3 PUFA in developing egg and larvae because of their preponderance in neural and visual tissues, which predominate in early stages of development.[39,40] Huynh, Kitts, Hu, and Trites (2007) showed that spawning herring exhibit a marked increase in the relative concentration of DHA, in milt and ovary.[41]

Despite these and many other previous studies that underlined the influences of spawning and season on PUFA content (especially ω3 PUFA) in edible muscle and organ tissues of different fish, there is a limited amount of information about the relationship between the F-acids' occurrence in fish tissues and the life cycle status of fish.

To acquire a deeper knowledge about this relationship, it is important to understand whether F-acids are functionally essential for normal growth, development, and reproduction in fishes. Moreover investigating the variation of F-acid amounts in edible muscle fish during the year may be convenient for nutritional purposes. Only a few studies reported that liver and testes lipids of salmonid fish contain high concentrations of F-acids, which vary seasonally, depending on spawning.[42−47]

As such, it seemed necessary to study the effect of spawning on changes in the fatty acid composition from different organs (ovaries, testes, brain, and eyes) and edible muscle of sardine and anchovy. The two species of fish were chosen based on their high nutritional value, availability year-round, and opposite spawning periods. Generally, in the Mediterranean Sea, the anchovy spawning period is constant from the end of April until early September, whereas the sardine-spawning period starts from October and lasts until April.[38,48−50]

For this purpose, the fatty acid profile of different tissues and edible muscle was determined by using capillary gas chromatography coupled with mass spectrometry (GC-MS). The fatty acids quantification was carried out using GC coupled with a flame ionization detector, GC-FID.

MATERIALS AND METHODS

Sampling. Samples were collected monthly from commercial landings of the midwater pelagic pair trawlers ("volante") fleet of Ancona (Central Adriatic Sea), from February 2010 to December 2010, excluding August, when fishery is not allowed in the Adriatic Sea due to fish reproduction. A total of 331 anchovies and 402 sardines were macroscopically sexed, analyzed, and used to estimate the monthly evolution of gonadosomatic index (GSI) and condition factor (CF). The total length (L_T, 0.5 cm accuracy), total body weight (W, 0.1 g accuracy), and gonad weights (W_G, 0.01 g accuracy) were also recorded.

Ten individuals for each species and for each sex were chosen monthly and were used to characterize the lipid fraction of the organ tissues and the edible muscle.

Biometric Measurements and Condition Indexes. To evaluate the reproductive status of fish was estimated the monthly evolution of GSI and CF. The relative robustness or degree of well being of a fish is expressed by condition factor. Variations of this coefficient primarily reflect the stage of development of the reproductive organs and consequentially reflect the different phases of the reproductive cycle.[51]

The GSI was calculated with eq 1 by expressing the monthly gonads weight average as proportion of the total body weight:

$$\mathrm{GSI} = 100 \times W_G/W \quad (1)$$

where W was gutted body weight and W_G was gonad weight.[52−54]

The condition factor was analyzed on a monthly basis according to eq 2:

$$\mathrm{CF} = W/a\,L^b \quad (2)$$

where a and b are the regression parameters of the length−weight relationship, W is body weight, and L is the total length.[52,55]

Extraction of Total Lipids. Ten individuals for each species and sex collected monthly were beheaded, eviscerated, and filleted without removing skin (edible muscle). Then the organ tissues such as brain, eyes, and gonads (ovaries and testes) were excised. An aliquot (10 g) of each organ tissues and edible muscle was homogenized in chloroform−methanol (1:2, v/v), and the lipids were extracted according to Bligh and Dyer (1959).[56]

Analysis of Fatty Acid Profile. Fatty acid methyl esters (FAMEs) were obtained from total lipids through alkaline transmethylation.[57] The qualitative analysis of FAMEs was carried out using a Focus gas chromatograph (Thermo Electron Corporation, West Palm Beach, FL, USA) equipped with a CP-Sil88 fused silica capillary column (100 m × 0.25 mm i.d., film thickness 0.2 μm, Chrompack, Middelburg, The Netherlands) and a quadrupole mass detector (FocusDSQ). The carrier gas was helium at a flow rate of 1.6 mL min^{-1}; the oven temperature program was 3 min at 55 °C, raised to 190 °C at a rate of 4 °C min^{-1}, then raised to 240 °C at a rate of 2 °C min^{-1}.The injector temperature was 230 °C. The sample was injected into a split/splitless system. The ion source temperature of the mass detector was set at 250 °C. The mass spectrum was acquired using Xcalibur Data System ver. 1.4. Peaks were identified by comparison with known standards and using the NIST mass spectral database.

The quantitative analysis of FAMEs was performed by means of gas chromatography using a CP-9002 apparatus (Chrompack, Middelburg, The Netherlands) equipped with a flame ionization detector (FID) and the same column and operative conditions reported above. The temperature of the detector was set at 240 °C. A Supelco (Bellefonte, PA, USA) standard solution containing a mixture of 37 FAMEs was used for identification of peaks and for the calculation of correction factor of the individual fatty acid peak areas. Fatty acid compositions (wt %) were calculated by the corrected peak area normalization method.

Statistical Analysis. The data collected from each monthly sampling were grouped into spawning and nonspawning samples, in order to point out the effect of spawning on lipid composition of edible muscle and organ tissues from the two fish species. The spawning season of each fish species was estimated according to GSI and CF measurements. Consequently, the data referring to spawning samples was calculated as the mean of data derived from fish caught during the estimated spawning season, whereas the data referring to nonspawning fish were calculated as the mean of data derived from fish caught during nonspawning season. All data were presented as group mean values ± standard deviation (SD).

The statistical analysis of data was performed by ANOVA carried out with the GraphPad InStat ver.3.0 system (GraphPad Software, San Diego, CA, USA). Tukey−Kramer's test was used for comparison of the means among the two groups of fish, spawning versus nonspawning, whereas pairwise differences were detected in order to get an overview of the differences in the fatty acid composition among the organ tissues. Significance was accepted at a probability of 0.01 ($p < 0.01$), according to the MSD (minimum significant differences) test.

RESULTS AND DISCUSSION

Condition Factor and Gonadosomatic Index for Sardine and Anchovy. Monthly trends of CF and GSI for sardine and anchovy are reported in Figure 1.

The analysis of the monthly average of the GSI showed a similar trend for both female and male sardines, so lastly we evaluated the monthly evolution of GSI for combined sexes. The sardine spawning period occurred from October to May,

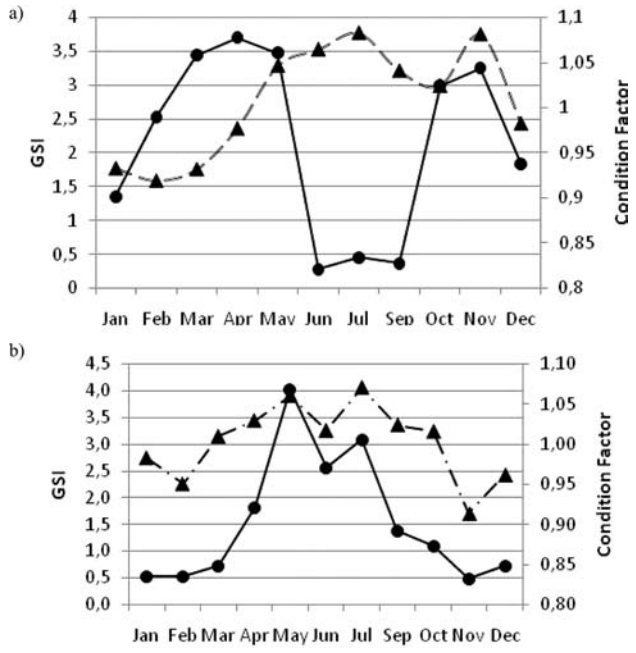

Figure 1. Monthly mean variations of condition factor, CF (▲), and gonadosomatic index, GSI (●), for (a) sardine (*Sardina pilchardus*) and (b) anchovy (*Engraulis encrasicolus*).

and the peak was observed from March to May, while the lowest values were recorded from June to September. Due to the absence of remarkable differences in the trend of monthly mean of the CF for males and females, the mean value of the condition factor for combined sexes (parameters estimated as a = 0.11035, b =2.85471, r = 0.95) was evaluated (Figure 1a). The temporal evolution of sardine CF showed two annual peaks (July and November) and the highest values from May to November, constituting the period of quiescence and the beginning of the reproductive season of sardine in the Adriatic Sea. The CF values decreased during the reproductive period, and this decrease could be explained by the energy reserves used for gonadic maturation and spawning instead of for maintenance and growth of individuals for which they are normally used, in agreement with other authors.[48,58−61] No correlation has been found (r^2 = 0.31174) between the trend of condition factor and the reproductive cycle.

Concerning the anchovy, the reproductive season ranged from April to September, and two peaks were observed: the first in May and the second, lower, in July. The trend of CF (parameters estimated as a = 0.004 06, b = 3.198 19, r = 0.97) showed two evident peaks during the year: one in May and the other in July (Figure 1b). This result could be related to greater food availability concentrated in those specific periods of the year and consequently to the annual cycle of primary production; similar results were found by Giraldez and Abad (1995), Millan (1999), and Regner (1996), where the fluctuations of the annual quantity of anchovy eggs coincided with fluctuations of primary production in the Adriatic Sea.[52,55,62] Moreover, we observed two peaks of the CF trend; the highest values were observed during the entire reproductive season, while the lowest values were obtained during the quiescence period. Differently from that reported for sardine, the anchovy cycle of CF showed a positive correlation with that of gonadosomatic index (r = 0.803 93).

Lipid Content and Fatty Acid Profile in Edible Muscle.

The lipid average, expressed on a wet weight basis, and the fatty acid composition (weight % of total fatty acids) of edible muscle (fillet) from spawning and nonspawning fish are reported in Table 1.

The highest lipid content ($p < 0.01$) was found in the edible muscle from nonspawning fish, for both sardine and anchovy. Taking into account the results obtained from GSI and CF measurements, the spawning seasons of the investigated sardine and anchovy were estimated from October to May (winter−spring) and from April to September (spring−summer), respectively. Thus, the sardine showed the highest fat content in the summer, whereas the anchovy was richer in fat in the winter. These findings agree with other studies that have described seasonal variation of fillet lipid content in different fishes (Zlatanos et al. 2007; Tufan et al. 2011; Luzia et al. 2003; Marin, Polak, Gasperlin, and Zlender, 2010).[9,34,38,63]

The qualitative fatty acid composition of edible muscle did not show any differences among the two species and the spawning condition. Twenty-seven fatty acids, including five congeners of F-acids, were identified in all fillets.

Differently, the fillet fatty acids profile showed remarkable differences in the individual fatty acid amounts. These differences were related to the spawning effect and to the fish species.

By and large, sardine was the species whose fatty acid composition of fillet lipids was most significantly affected by the spawning season. The spawning effect led to an increase in polyunsaturated fatty acids (PUFA), with a simultaneous decrease of monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA), on fatty acid balance of fillets form spawning sardine and anchovy.

In detail, the amount of fatty acid classes in all fish fillets increased in the order F-acids < MUFA < SFA < PUFA.

The abundance of an F-acids fraction, expressed as % of total fatty acids, was lower than 1% in all fish fillets. The identified F-acids were 10,13-epoxy-11-methyloctadeca-10,12-dienoic [MonoMe(9,5)], 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic [DiMe(9,5)], 12,15-epoxy-13-methyleicosa-12,14-dienoic [MonoMe(11,5)], 12,15-epoxy-13,14-dimethyloctadeca-12,14-dienoic [DiMe(11,3)], and 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic [DiMe(11,5)] in all the samples. The spawning season had no effect on F-acid fraction composition for both sardine and anchovy.

The MUFA portion constituted nearly one-quarter of the total fatty acids, with oleic acid (C18:1Δ^{9cis}) as the predominant MUFA in all fish fillets. A higher level of MUFA occurred in all fillets from nonspawning fish with respect to spawning fish even if this MUFA variation was significant only for the sardine, and it was due to the significant increase of C18:1Δ^{9cis}, eicosamonoenoic (C20:1Δ^{11cis}), and palmitoleic (C16:1Δ^{9cis}) acids.

The SFA represented one-third of total fatty acids, with palmitic acid (C16:0) being the most concentrated, in all fish fillets. Fluctuations in SFA content can be observed by comparing the fillet from spawning and nonspawning fish. The highest average content of SFA was displayed in edible muscle from nonspawning fish, for both sardine and anchovy. Similar to MUFA, the SFA variation was significant ($p < 0.01$) only for sardine (32.9 ± 1.8 vs 35.3 ± 1.3), where it was a primary consequence of the miristic (C14:0) and arachidic (C20:0) acids' increase and of the palmitic acid reduction. This is consistent with a previous study (Garrido et al. 2007) that

Table 1. Lipid Content and Fatty Acid Composition (weight % of total fatty acids) of the Edible Muscle from Spawning and Nonspawning Fish[a]

	sardine		enchovy	
	spawning (n = 7)	nonspawning (n = 3)	spawning (n = 5)	nonspawning (n = 5)
lipid content (%)	0.6 ± 0.2*	1.5 ± 0.4*	0.6 ± 0.3*	1.3 ± 0.4*
fatty acid (%)				
C14:0	1.7 ± 0.5*	5.6 ± 0.6*	3.3 ± 0.9	4.0 ± 1.2
C15:0	0.6 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.2
C16:0	24.5 ± 1.1*	21.6 ± 1.0*	22.4 ± 1.8	22.1 ± 0.8
C17:0	0.8 ± 0.3	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.2
C18:0	4.8 ± 0.9	5.0 ± 0.4	4.6 ± 0.5	4.8 ± 0.6
C20:0	0.1 ± 0.1*	1.0 ± 0.4*	0.4 ± 0.4	0.3 ± 0.2
C24:0	0.4 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
Tot SFA	32.9 ± 1.8*	35.3 ± 1.3*	33.1 ± 1.1	32.6 ± 1.5
C16:1 Δ9	1.9 ± 0.6*	4.4 ± 1.0*	2.3 ± 0.7	2.9 ± 0.7
Iso C16:1	0.3 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
C17:1	0.3 ± 0.1*	0.5 ± 0.2*	0.5 ± 0.1	0.5 ± 0.0
C18:1 Δ9*trans*	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
C18:1 Δ9*cis*	5.1 ± 0.7*	8.5 ± 1.4*	5.4 ± 1.2	5.5 ± 0.4
C18:1 Δ11	2.2 ± 0.3	2.3 ± 0.2	2.3 ± 0.3	2.9 ± 0.4
C20:1 Δ11	0.8 ± 0.3*	1.2 ± 0.2*	0.7 ± 0.2	0.9 ± 0.5
C24:1	1.1 ± 0.4	1.0 ± 0.3	0.2 ± 0.1	0.2 ± 0.1
Tot MUFA	12.0 ± 1.2*	18.4 ± 2.5*	12.1 ± 1.6	13.7 ± 1.1
C18:2 Δ9,12ω6	0.8 ± 0.4*	1.7 ± 0.1*	1.4 ± 0.2	1.3 ± 0.2
C18:3 Δ9,12,15ω3	0.3 ± 0.2*	1.4 ± 0.3*	0.8 ± 0.5	0.9 ± 0.3
C18:4 6,9,12,15ω3	0.9 ± 0.4*	2.9 ± 0.5*	1.2 ± 0.6	1.3 ± 0.4
C20:4 Δ5,8,11,14ω6	1.4 ± 0.2	1.1 ± 0.4	1.3 ± 0.5	1.8 ± 0.3
C20:5 Δ5,8,11,14,17ω3	7.2 ± 2.2	9.4 ± 0.7	7.0 ± 1.4*	9.3 ± 2.3*
C22:5 Δ7,10,13,16,19ω3	1.1 ± 0.2	1.2 ± 0.2	0.8 ± 0.2	1.0 ± 0.1
C22:6 Δ4,7,10,13,16,19ω3	38.9 ± 4.1*	24.1 ± 2.2*	36.3 ± 3.6*	29.7 ± 3.4*
Tot PUFAω3	48.5 ± 2.6*	39.2 ± 1.9*	46.3 ± 1.9*	42.4 ± 0.6*
Tot PUFAω6	2.4 ± 0.3*	3.0 ± 0.4*	3.1 ± 0.4	3.5 ± 0.5
MonoMe (9,5)	0.2 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
MonoMe (11,5)	0.1 ± 0.1	tr	0.2 ± 0.1	0.1 ± 0.1
DiMe (9,5)	tr	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
DiMe (11,3)	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
DiMe (11,5)	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1
Tot F-acids	0.5 ± 0.2	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.1

[a]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; F-acids, furan fatty acids. Results represent means ± SD (n = number of sampling corresponding to spawning or nonspawning season of each fish species). Pairs of means corresponding to spawning and nonspawning samples were compared, and those that were significantly different ($p < 0.01$) are identified by (*); tr, lower than 0.1%. Cm:n Δx; m = number of carbon atoms, n = number of double bonds, x = position of double bonds. MonoMe (p,q), 13-methyl furan fatty acid with p carbon atoms in the carboxyl side and q carbon atoms in the alkyl side.

showed that C16:0 content decreased sharply from fillets of Iberian sardine caught in February (end of spawning season) to sardine fillets caught in November (beginning of spawning season).[39]

The PUFA portion accounted for 42−51% of total fatty acids in different fillets. DHA was found to be the most abundant fatty acid as well as the main PUFA in all samples. The DHA level was significantly ($p < 0.01$) higher in all spawning fillets with respect to nonspawning fillets. The EPA content also was higher in all nonspawning fillets, but this difference was proven statistically significant only for the anchovy. The ratio ω3 PUFA/ω6 PUFA decreased on passing from spawning to nonspawning fillets, for both sardine and anchovy. Thus, the sardine and anchovy fillets from spawning fish had a higher nutritional value than nonspawning fillets, since the ω3/ω6 ratio has been suggested to be a useful indicator for comparing relative nutritional values of fish products. These findings were in good agreement with published fatty acid data of Mediterranean fish. For example, Zlatanos et al. (2007) reported that anchovy exhibited the highest values of ω3 PUFA in the months of April and June (spring season; corresponding to anchovy spawning season estimated by us), whereas sardine has the highest ω3 PUFA content in the months of October, December, and February (autumn/winter season; corresponding to sardine spawning season).[34] Pirini et al. (2010) studied the effect of the catching season (either autumn/winter or spring) on lipid content and fatty acid profile of anchovy, sardine, sprat, and horse mackerel from the North Adriatic Sea.[32] They reported that the fatty acid composition most profoundly affected by the season of catch was that of anchovy, autumn batches being richer in the most important SFA and ω6 PUFA, and spring batches richer in EPA and DHA. Sardine lipids seem to be less affected by the season of catch than were those from anchovy. The last observation is seemingly discordant with our set of results, which clearly indicated that spawning season has a greater effect on the fatty

Table 2. Fatty Acid Composition (weight % of total fatty acids) of Organ Tissues (brain, eye, ovaries, and testes) from Spawning and Nonspawning Sardine[a]

	brain (1)		eyes (2)		ovaries (3)		testes (4)		pairwise differences $p < 0.01$					
fatty acid (%)	spawning ($n = 7$)	nonspawning ($n = 3$)	spawning ($n = 7$)	nonspawning ($n = 3$)	spawning ($n = 7$)	nonspawning ($n = 3$)	spawning ($n = 7$)	nonspawning ($n = 3$)	1−2	1−3	1−4	2−3	2−4	3−4
C14:0	0.7 ± 0.3	1.4 ± 0.4	2.8 ± 0.7	3.2 ± 1.0	2.2 ± 0.4*	5.9 ± 2.2*	1.8 ± 0.8*	5.4 ± 0.2*	○	●	●	●		
C15:0	0.3 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.5 ± 0.3*	1.0 ± 0.1*		○●	●	●		
C16:0	15.5 ± 1.7	15.8 ± 1.0	22.5 ± 2.7	18.5 ± 0.4	23.5 ± 0.8	23.5 ± 0.1	26.2 ± 2.4	24.1 ± 3.9	○	○●	○●			
C17:0	0.6 ± 0.2	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	1.2 ± 0.2	1.4 ± 0.3	0.7 ± 0.3	1.3 ± 0.3		○●	●	●	○	
C18:0	7.2 ± 0.8	6.8 ± 0.5	8.7 ± 0.4*	6.9 ± 0.7*	3.9 ± 1.8	5.0 ± 0.3	4.2 ± 1.3	5.5 ± 1.1	○	○	○	○	○	
C20:0	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.1 ± 0.1*	1.6 ± 0.6*	0.1 ± 0.1*	1.3 ± 0.0*		●	●	●	●	
C24:0	3.5 ± 1.1	2.7 ± 0.5	1.4 ± 0.8	1.1 ± 0.4	0.3 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.6 ± 0.4	○●	○●	○●			
Tot SFA	28.3 ± 2.8	28.5 ± 2.4	37.8 ± 3.6	31.8 ± 0.7	32.1 ± 1.3	38.9 ± 1.4	33.9 ± 4.8	39.3 ± 4.8	○	●	●			
C16:1 Δ9	3.7 ± 1.1	4.1 ± 0.3	4.6 ± 1.0	4.2 ± 0.4	4.1 ± 1.6	5.4 ± 0.2	2.0 ± 0.5	4.5 ± 0.7					○	
Iso C16:1	0.6 ± 0.2	0.6 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.4	0.4 ± 0.2	0.6 ± 0.3	0.4 ± 0.0						
C17:1	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.2	0.7 ± 0.1						
C18:1 Δ9 *trans*	0.1 ± 0.1	0.1 ± 0.0	tr	tr	0.1 ± 0.1	0.3 ± 0.0	tr	tr						
C18:1 Δ9 *cis*	29.3 ± 1.6	26.1 ± 2.2	12.6 ± 2.9	12.4 ± 2.4	6.0 ± 0.8	9.2 ± 1.0	6.3 ± 1.2	9.6 ± 0.3	○●	○●	○●	○	○	
C18:1 Δ11	1.7 ± 0.6	1.7 ± 0.2	3.0 ± 0.5	2.6 ± 0.5	2.3 ± 0.6	3.2 ± 0.4	4.0 ± 0.8	2.7 ± 0.0	○	●	○			○
C20:1 Δ11	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.3	0.7 ± 0.1	0.6 ± 0.3*	1.3 ± 0.3*	0.5 ± 0.1*	1.2 ± 0.0*	○●		○	○●	○●	
Iso C20:1	tr	tr	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	tr	0.1 ± 0.0	0.2 ± 0.0						
C24:1 Δ9	5.1 ± 1.9	5.8 ± 1.1	1.8 ± 0.9	2.1 ± 0.5	0.8 ± 0.2	0.6 ± 0.2	tr	0.2 ± 0.0	○●	○●	○●			
Tot MUFA	42.3 ± 1.2	39.9 ± 2.2	23.9 ± 3.5	23.3 ± 3.0	15.4 ± 1.3	21.2 ± 1.3	14.3 ± 1.9	20.4 ± 0.0	○●	○●	○●	○	○	
C18:2 Δ9,12ω6	0.2 ± 0.1	0.5 ± 0.2	0.7 ± 0.2	1.0 ± 0.4	1.0 ± 0.2*	2.0 ± 0.4*	1.0 ± 0.1*	2.1 ± 0.1*		○●	○●	●	●	
C18:3 Δ6,9,12ω6	tr	tr	tr	tr	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0						
C18:3 Δ9,12,15ω3	0.2 ± 0.2	0.4 ± 0.1	0.2 ± 0.1*	0.8 ± 0.3*	0.6 ± 0.2*	1.5 ± 0.5*	0.4 ± 0.2*	1.7 ± 0.1*		●	●	●	●	
C18:4 6,9,12,15ω3	0.3 ± 0.1	0.6 ± 0.2	0.5 ± 0.1*	1.5 ± 0.3*	0.7 ± 0.2*	2.5 ± 0.9*	0.5 ± 0.1*	2.9 ± 0.3*	●	●	●	●	●	
C20:2 Δ11,14ω6	tr	tr	n.d	n.d	0.2 ± 0.1	n.d	tr	n.d						
C20:4 Δ5,8,11,14ω6	1.2 ± 0.5	1.4 ± 0.3	1.3 ± 0.4	1.4 ± 0.3	1.8 ± 0.7	1.4 ± 0.5	2.1 ± 0.3	1.1 ± 0.1						
C20:5 Δ5,8,11,14,17ω3	3.7 ± 0.5	3.9 ± 0.7	3.8 ± 0.7	5.5 ± 1.4	10.8 ± 2.2	8.2 ± 2.1	9.5 ± 2.0	8.3 ± 2.4		○●	○●	○	○	
C22:5 Δ7,10,13,16,19ω3	0.8 ± 0.2	0.7 ± 0.1	0.6 ± 0.2	0.8 ± 0.1	1.6 ± 0.3	1.1 ± 0.1	1.1 ± 0.2	1.0 ± 0.3		○		○	○	
C22:6 Δ4,7,10,13,16,19ω3	17.4 ± 2.3	17.0 ± 0.7	27.0 ± 3.8	28.7 ± 2.6	30.1 ± 2.9*	17.4 ± 3.6*	33.0 ± 3.8*	17.6 ± 2.3*	○●	○	○	●	●	
Tot PUFAω3	22.8 ± 2.7	23.3 ± 1.4	32.1 ± 4.5	37.6 ± 3.3	43.9 ± 1.7*	30.8 ± 2.9*	44.8 ± 6.1*	32.2 ± 5.7*	○●	○	○	○	○	
Tot PUFAω6	1.5 ± 0.7	2.0 ± 0.4	2.2 ± 0.6	2.5 ± 0.5	3.2 ± 0.5	3.6 ± 0.6	3.3 ± 0.5	3.3 ± 0.1		○●	○			
MonoMe (9,5)	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	tr*	0.5 ± 0.1*		●	●			
MonoMe (11,5)	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	n.d	n.d	0.2 ± 0.1	n.d						
DiMe (9,5)	n.d	n.d	0.1 ± 0.1	tr	tr	0.1 ± 0.1	tr	0.1 ± 0.0						
DiMe (11,3)	0.2 ± 0.2	0.5 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.4 ± 0.3	●	●				
DiMe (11,5)	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	tr	0.1 ± 0.1	tr	tr						
Tot F-acids	0.6 ± 0.2	0.9 ± 0.1	0.6 ± 0.2	0.5 ± 0.3	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.3	1.0 ± 0.4						

[a]The asterisk (*) underlines statistical differences among fatty acid compositions of spawning and nonspawning samples. The cases where the level of fatty acids was different with a $p < 0.01$ between the organs derived from fish with the same reproductive status are shown with ○ for spawning season and ● for nonspawning season. tr, lower than 0.1%. n.d, not detectable.

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Table 3. Fatty Acid Composition (weight % of total fatty acids) of Organ Tissues (brain, eye, ovaries, and testes) from Spawning and Nonspawning Anchovy[a]

	brain (1)		eyes (2)		ovaries (3)		testes (4)		pairwise differences $P < 0.01$					
fatty acid	spawning ($n = 5$)	nonspawning ($n = 5$)	spawning ($n = 5$)	nonspawning ($n = 5$)	spawning ($n = 5$)	nonspawning ($n = 5$)	spawning ($n = 5$)	nonspawning ($n = 5$)	1−2	1−3	1−4	2−3	2−4	3−4
C14:0	1.3 ± 0.5	1.4 ± 0.4	2.7 ± 0.9	2.8 ± 0.7	2.2 ± 0.6*	5.4 ± 1.3*	2.9 ± 1.7*	7.6 ± 1.2*		●	○●		●	
C15:0	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	05 ± 0.2	0.9 ± 0.3	0.9 ± 0.1	0.7 ± 0.2	1.3 ± 0.4			○●		●	
C16:0	13.2 ± 0.9	13.0 ± 1.0	17.7 ± 2.9	20.4 ± 1.6	25.8 ± 2.6	26.9 ± 1.6	26.9 ± 2.0	22.4 ± 1.0	○●	○●	○●	○●	○	
C17:0	0.8 ± 0.2	0.8 ± 0.2	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	1.4 ± 0.4	0.7 ± 0.3	1.2 ± 0.4						
C18:0	6.6 ± 0.6	6.9 ± 0.8	7.7 ± 0.6	8.1 ± 0.9	4.8 ± 1.0	4.9 ± 1.5	4.5 ± 0.5	5.1 ± 0.2				○●	○●	
C20:0	0.3 ± 0.2	0.3 ± 0.1	0.2 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.7 ± 0.1						
C24:0	4.9 ± 0.8	4.6 ± 0.9	1.5 ± 0.9	1.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	○●	○●	○●			
Tot SFA	28.1 ± 1.1	28.2 ± 2.4	29.4 ± 3.7	33.2 ± 2.9	34.8 ± 1.1	40.2 ± 2.4	35.9 ± 1.7	38.5 ± 0.8		○●	○●		○	
C16:1 Δ9	5.3 ± 0.6	5.4 ± 1.0	3.3 ± 0.8	4.4 ± 0.7	1.9 ± 0.6*	4.5 ± 0.3*	1.7 ± 0.6*	5.2 ± 0.5*	○	○	○		○	
Iso C16:1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.0						
C17:1	1.0 ± 0.1	0.8 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.6 ± 0.2	○	○	○			
C18:1 Δ9*trans*	0.1 ± 0.0	tr	0.0 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.2						
C18:1 Δ9*cis*	24.6 ± 2.8	25.2 ± 2.2	10.2 ± 1.8	12.0 ± 1.8	6.8 ± 1.0	7.3 ± 0.7	7.5 ± 1.9	8.6 ± 0.5	○●	○●	○●			
C18:1 Δ11	1.8 ± 0.4	1.7 ± 0.2	2.1 ± 0.3	2.5 ± 0.4	1.5 ± 0.5	2.6 ± 0.7	3.6 ± 0.7	3.0 ± 0.8			○	○		○
C20:1 Δ11	0.6 ± 0.1	0.5 ± 0.0	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.1	1.2 ± 0.4	0.6 ± 0.1	1.2 ± 0.1						
Iso C20:1	0.1 ± 0.1	tr	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.4 ± 0.1						
C24:1 Δ9	4.8 ± 1.1	5.0 ± 1.2	0.9 ± 0.1	1.3 ± 0.1	0.2 ± 0.0	0.6 ± 0.0	0.3 ± 0.2	0.7 ± 0.1	○●	○●	○●			
Tot MUFA	38.8 ± 3.3	39.1 ± 2.1	18.4 ± 2.6	22.0 ± 2.4	11.9 ± 1.3	18.1 ± 0.9	14.9 ± 0.7	20.8 ± 0.9	○●	○●	○●	○		
C18:2 Δ9,12ω6	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	1.5 ± 0.2	2.0 ± 0.4	1.4 ± 0.2	1.9 ± 0.1		○●	○●	○●	○●	
C18:3 Δ6,9,12ω6	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	tr	0.1 ± 0.1						
C18:3 Δ9,12,15ω3	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2*	0.9 ± 0.2*	0.7 ± 0.2*	1.0 ± 0.1*		●	○●	●	○●	
C18:4 6,9,12,15ω3	0.6 ± 0.2	0.5 ± 0.2	0.6 ± 0.3	0.5 ± 0.0	0.7 ± 0.1*	1.7 ± 0.1*	0.7 ± 0.2*	1.9 ± 0.4*		●	●	●	●	
C20:2 Δ11,14ω6	tr	tr	tr	tr	0.1 ± 0.1	0.2 ± 0.1	tr	0.3 ± 0.0						
C20:4 Δ5,8,11,14ω6	2.0 ± 0.3	2.2 ± 0.2	1.8 ± 0.7	1.5 ± 0.4	1.9 ± 0.9	1.4 ± 0.3	1.1 ± 0.2	1.5 ± 0.1						
C20:5 Δ5,8,11,14,17ω3	4.3 ± 0.7	4.8 ± 0.5	4.3 ± 1.0	4.0 ± 0.7	10.0 ± 1.1	7.9 ± 1.8	7.8 ± 1.5	8.5 ± 1.5		○●	○●	○●	○●	○
C22:5 Δ7,10,13,16,19ω3	0.9 ± 0.1	1.0 ± 0.1	0.7 ± 0.2	0.6 ± 0.2	1.2 ± 0.4	1.0 ± 0.3	0.9 ± 0.3	1.0 ± 0.2				○		
C22:6 Δ4,7,10,13,16,19ω3	17.9 ± 0.8	17.7 ± 1.6	34.9 ± 4.6	29.8 ± 2.6	31.7 ± 2.4*	17.5 ± 2.7*	31.6 ± 2.6*	15.4 ± 2.4*	○●	○	○	●	●	○●
Tot PUFAω3	24.4 ± 1.5	24.7 ± 1.1	35.3 ± 2.9	35.3 ± 2.9	44.2 ± 3.7*	29.2 ± 2.8*	41.9 ± 2.0*	28.0 ± 0.8*	○●	○	○			
Tot PUFAω6	2.9 ± 0.5	2.8 ± 0.3	2.5 ± 0.4	2.3 ± 0.4	3.7 ± 0.9	3.7 ± 0.6	2.6 ± 0.3	3.9 ± 1.0						
MonoMe (9,5)	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.0						
MonoMe (11,5)	0.2 ± 0.1	tr	0.3 ± 0.1	0.2 ± 0.2	tr	tr	tr	tr						
DiMe (9,5)	n.d	n.d	0.1 ± 0.1	tr	tr	0.1 ± 0.1	tr	0.1 ± 0.0						
DiMe (11,3)	0.2 ± 0.0	0.1 ± 0.1	0.5 ± 0.3	0.7 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.3	0.1 ± 0.1						
DiMe (11,5)	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.1						
Tot F-acids	0.8 ± 0.2	0.6 ± 0.2	0.9 ± 0.2	0.9 ± 0.4	0.6 ± 0.1	0.6 ± 0.2	0.8 ± 0.3	0.5 ± 0.2						

[a]The asterisk (*) underlines statistical difference among fatty acid composition of spawning and nonspawning samples. The cases where the level of fatty acids were different on a $p < 0.01$ between the organs deriving from fish with the same reproductive status are shown with ○ for spawning season and ● for nonspawning season. *tr*, lower than 0.1%. n.d, not detectable.

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acid composition of sardine fillets rather than those of anchovy (Table 1). The discrepancy between our data concerning the sardine composition and Pirini et al.'s (2010) results can be explained taking into account that Pirini et al. performed the sampling of fish during autumn/winter and spring.[32] These seasons coincided exclusively with the sardine spawning season (estimated by us), but not with nonspawning season (estimated by us), which matched with summer. Thus, the modification detected by Pirini et al. can be related to different feeding conditions, whereas the changes reveled in our data can be related to the combined effect of spawning and feeding conditions. On the whole, the results suggest that changes in fatty acid composition of fish fillets seem to be more dependent on their reproductive stage than on their feeding conditions.

Fatty Acid Profile of the Organ Tissues. The fatty acid compositions of total lipids from different organ tissues of sardine and anchovy, together with significant differences ($p < 0.01$) among the samples, are presented in Tables 2 and 3, respectively. Thirty fatty acids, including five congeners of F-acids, were found.

The fatty acid profile exhibited many differences in the relative distribution of individual fatty acids among organ tissues and between spawning and nonspawning fish. Differently, no variation was revealed from the comparison of the fatty acid composition of sardine and that of anchovy organ tissues.

By and large, the spawning effect gave rise to the same trend of organ tissues' fatty acid variation, for both sardine and anchovy. Palmitic acid was a primary SFA in all organ tissues, and its abundance was not affected by the spawning status of fish. Contrarily, an increasing trend in the amount of C14:0 in ovaries and testes lipid was reported for fish during nonspawning time. Noticeable was the significant highest content of tetracosanoic acid (C24:0) exhibited by all brain samples with respect to all other organ tissues.

MUFA constituted nearly half of the total fatty acids in brain lipids of sardine and anchovy but less than one-quarter in eyes, ovaries, and testes. MUFA dominated brain lipids of both spawning and nonspawning fish due to the abundance of C18:1 Δ^{9cis}, which accounted for about 25% of the total fatty acid in all fish brain. This C18:1 level was significantly higher ($p < 0.01$) than that detected in all other organ tissues; thus oleic acid became the most abundant fatty acid of the fish brain lipids and the major fatty acid of the MUFA fraction in all organ tissues. As expected, the brain tissues were significantly richer in nervonic (C24:1 Δ^9) acid with respect to all other organ tissues.

Another interesting feature of the MUFA was the decrease of C16:1 Δ^9 and C20:1 Δ^{11} concentration from nonspawning to spawning in ovaries and testes, reported also in edible muscle. The C16:1 Δ^9 decrease was statistically significant for the anchovy but not for the sardine, whereas the C20:1 Δ^{11} decrease was significant only for the sardine. The influence of spawning period on C20:1 Δ^{11} concentration was in agreement with previous studies,[38,39,41] which showed that spawning fish require C20:1 Δ^{11} for energy metabolism during the course of gonad development.

The percentage contribution of ω3 PUFA exhibited a variation related to different organ tissues and to spawning period, for both sardine and anchovy. DHA was found to be the major ω3 PUFA as well as the main fatty acid in all eye samples and in gonads from spawning fish. As a consequence, all eye and gonad tissues from spawning fish were significantly richer in DHA when compared to all other samples. EPA was found to be a second major ω3 PUFA with significant changes among organs but without important variation related to spawning period. Thus, the statistically highest EPA value occurred in all gonads (from 8.2% ± 2.1 to 10.8% ± 2.2 for sardine and from 7.8 ± 1.5 to 10.0 ± 1.1% for anchovy) and the lowest in all brain and eye tissues, for both sardine and anchovy. Although EPA content weakly increased from gonads of nonspawning to those of spawning fish, this increase was not statistically significant ($p > 0.01$). The comparison of our findings with those reported in literature become somewhat difficult, since there is no existing research on fatty acid values representing gonads of anchovy and sardine caught from the Adriatic and/or Mediterranean Sea. Anyway, our data were in agreement with a previous study,[41] which pointed out that spawning Pacific herring had significantly higher PUFA content in the milt and ovary, with DHA having the greatest proportion.

The converse trends of variation were recovered for α-linolenic (C18:3 ω3) and octatetraenoic (C18:4 ω3) acids, which were significantly higher in the ovaries and testes from nonspawning than in all the other samples, including the gonads from spawning fish. Thus, unlike DHA, the spawning time led to a decrease of these ω3 PUFA in the gonads.

The proportions of ω6 PUFA were relatively low in all samples (<4% of total fatty acids). Arachidonic acid (C20:4 ω6) was the most representative ω6 PUFA in all samples, except in gonads from nonspawning sardine and anchovy, where the main ω6 PUFA was linoleic acid (C18:2 ω6). The C18:2 ω6 enrichment of ovaries and testes from nonspawning fish was statistically significant for the sardine, but not for anchovy.

Similar to edible muscle samples, the F-acid profile was not affected by the spawning season, while it exhibited differences among the organ tissues. In detail, the F-acid fraction accounted for less than 1% of total fatty acids in all organ tissues, for both spawning and nonspawning fish. The F-acid fractions of eyes, ovaries, and testicles from spawning and nonspawning sardine and anchovy were composed by following F-acids MonoMe(9,5), DiMe(9,5), MonoMe(11,5), DiMe(11,3), and DiMe(11,5). Otherwise the F-acid fractions of all brain samples did not display DiMe(9,5).

In conclusion our results provide new evidence concerning the spawning effect on the F-acid distribution in edible muscle and organ tissues (brain, eye, and gonad) of pelagic fish, such as anchovy and sardine. The data indicated that the F-acid profile of edible muscle, brain, eye, and gonad of anchovy and sardine was not affected by the spawning season but exhibited differences among the organ tissues. The F-acid fraction of the brain was markedly different from all the other tissues and from the edible muscle, since it was lacking the DiMe(9,5). The fact that the concentration of F-acids in gonads from spawning fish remained similar to those in gonads from nonspawning fish suggests that the F-acids are not involved in the reproduction function.

Unlike the F-acids, the whole fatty acid profile of edible muscle and gonads of anchovy and sardine was strongly affected by the spawning season, which led to the increase of PUFA, specifically of DHA, and the simultaneous decrease of MUFA and SFA on fatty acid balance of edible muscle and gonads from spawning fish. Furthermore, the gonads from spawning fish were richer in DHA and C20:4 ω6, while the gonads from nonspawning fish were richer in their precursor, such as α-linolenic (C18:3 ω3) and linoleic (C18:2 ω6) acid.

These marked fatty acid variations seem to be more dependent on the reproductive stage of the fish rather than their feeding conditions, since identical trends of changes occurred in both anchovy and sardine, which are characterized by the same feeding but opposite spawning seasons. Additionally, the DHA enrichment of edible muscle and gonads from spawning fish confirms that DHA is functionally essential for normal growth, development, and reproduction in fish. The same observation has been reported for Mediterranean sardines and for anchovy caught in the Turkish Black Sea.[38] From a nutritional point of view and according to the fact that the $\omega3/\omega6$ ratio has been suggested to be a useful indicator for comparing the relative nutritional value of fish products, it is possible to state that sardine and anchovy fillets from spawning fish have a higher nutritional value than nonspawning fillets, since the ratio $\omega3$ PUFA/$\omega6$ PUFA decreases between spawning and nonspawning fillets.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

ANOVA, analysis of variance; CF, condition factor; DHA, docosahexaenoic acid; DiMe(9,5), 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic; DiMe(11,3), 12,15-epoxy-13,14-dimethyloctadeca-12,14-dienoic; DiMe(11,5), 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; F-acids, furan fatty acids; FAME, fatty acid methyl ester; GC-FID, gas chromatography coupled with flame ionization detector; GC-MS, gas chromatography coupled with mass spectrometry; GSI, gonadosomatic index; W_G, gonad weights; MonoMe(9,5), 10,13-epoxy-11-methyloctadeca-10,12-dienoic; MonoMe(11,5), 12,15-epoxy-13-methyleicosa-12,14-dienoic; MSD, minimum significant difference; MUFA, monounsaturated fatty acids; NIST, National Institute of Standards and Technology; PUFA, polyunsaturated fatty acids; SAT, saturated fatty acid; SD, standard deviation; SFA, saturated fatty acid; L_T, total length; W, total body weight

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