Composition and Seasonal Variation of Fatty Acids of *Diplodus vulgaris* L. from the Adriatic Sea

Jadranka Varljen[a,*], Lara Baticic[a], Gordana Sincic-Modric[a], Vojko Obersnel[b], and Miljenko Kapovic[b]

Departments of [a]Chemistry and Biochemistry and [b]Biology, Faculty of Medicine, University of Rijeka, 51000 Rijeka, Croatia

ABSTRACT: Muscle tissue from the common two-banded sea bream *Diplodus vulgaris* L. originating from the Adriatic Sea, Croatia, was analyzed. The FA composition of neutral (TAG) and polar (PE, PC, PI/PS) lipid classes was determined, as well as the lipid and water contents during winter and summer periods. Both the total lipid and water contents were higher in the winter period. We identified 16 different FA. The major constituents of the total FA in both seasons were saturates: palmitic (16:0) and stearic acids (18:0); monoenes: oleic (18:1n-9) and palmitoleic acids (16:1n-7); and polyunsaturates: arachidonic acid (20:4n-6), EPA (20:5n-3), and DHA (22:6n-3), but their amounts and ratios differed significantly between the two seasons and between lipid fractions. The FA composition showed a noticeable pattern of seasonality that reflected fluctuations mainly in TAG. The diminution of the monounsaturated FA content in the summer was clearly followed by an increase in PUFA content. *Diplodus vulgaris* is a good source of natural n-3 PUFA and would therefore be suitable for inclusion in highly unsaturated low-fat diets.

Paper no. J10714 in *JAOCS 81,* 759–763 (August 2004).

KEY WORDS: Adriatic Sea, *Diplodus vulgaris*, fatty acid composition, fish, lipids, seasonal variation, two-banded sea bream.

Scientific evidence clearly shows that dietary FA composition is involved in the etiology of many diseases. In recent years, the study of n-3 PUFA in marine foods has been emphasized because of their beneficial effects on cardiovascular diseases, which are the main cause of death in occidental countries, as described in the literature (1). Increasing evidence suggests that n-3 FA derived from fish and fish oils, particularly EPA (20:5n-3) and DHA (22:6n-3), may play a protective role in coronary artery disease and its many complications through a variety of actions, including controlling the levels of blood lipids, blood pressure, cardiac and vascular function, and coagulation (2,3). Epidemiological studies have demonstrated that the incidence of coronary artery disease is inversely associated with consumption of n-3 PUFA. Therefore, the nutritional importance of fish consumption is associated largely with the n-3 FA content (4). On the basis of our current knowledge, the recommendation, particularly for high-risk populations, to increase the dietary intake of n-3 PUFA through the consumption of fish is justified.

Because of those findings, many dietary supplements, pharmaceuticals, and other products based on fish oil FA have been developed and produced commercially (5). Thus, there is a growing requirement for marine fish lipids. Therefore, nutritionists and food scientists need FA compositional data for dietary formulation, processing, and product development. FA compositional data for a great number of marine, freshwater, and farmed fish species originating from different parts of the world are available in the literature (e.g., Ref. 6).

Lipid content and FA composition vary not only between fish species but also within a species as a function of such factors as fish age, gender, nutritional habits, water temperature, and season (4). Published information about the FA composition of Adriatic Sea fish species and the influence on it of different eco-physical parameters is scant.

We investigated the composition and the seasonal variation of FA in a commercially important fish species in the Adriatic Sea, the two-banded seabream (*Diplodus vulgaris* L.). The FA composition of neutral (TAG) and polar (PE, PC, PI/PS) lipid classes was determined, as well as the lipid and water contents in fish muscle tissue samples.

EXPERIMENTAL PROCEDURES

Collection of fish species. Samples of two-banded sea bream (*Diplodus vulgaris* L.) were collected from the Kvarner Bay in the Adriatic Sea, Croatia; five specimens were collected twice during late winter (from February to March 2002) and five specimens twice in late summer (from August to September 2002), forming two groups of 10 fish. Fish were captured by long-line at a depth of 10–15 m, overnight. Ten live specimens of similar body weight and length were selected from all the captured specimens. Biological characteristics were determined after every collection. Body weight (g) and length (cm) were noted, and fish were dissected immediately after catch.

Prior to analysis, samples of about 5 g of white fish muscle were taken from the middle of the left lateral region of the body for the determination of FA composition. Likewise, samples of about 1 g of muscle tissue were set aside for water content analysis (see next paragraph). Each sample was put

*To whom correspondence should be addressed at Department of Chemistry and Biochemistry, Faculty of Medicine, University of Rijeka, Brace Branhetta 20, 51000 Rijeka, Croatia. E-mail: vjadran@medri.hr

Present address of fourth author: City of Rijeka, Korzo 16, 51000 Rijeka, Croatia.

into a plastic tube, sealed, and marked, then transported on ice to the laboratory of the Department of Chemistry and Biochemistry at the Faculty of Medicine, Rijeka. Fish muscle tissue samples were preserved at –20°C for further analysis.

Water content analysis. The water content was determined in fish muscle tissue samples having an average mass of 1 g. The samples, that were separated for water content analysis were preserved overnight at +4°C and analyzed immediately the following day, so as to obtain reliable results. The analyses were performed after drying the tissue at 105°C to a constant mass.

Extraction of total lipids. Total lipids were extracted from fish muscle tissue samples according to Folch *et al.* (7). A chloroform/methanol solvent mixture (2:1, vol/vol) was added to frozen samples in the ratio solvent/tissue of 20:1 (vol/wt). The samples were homogenized three times for 10 min at 3000–4000 rpm. Each homogenization step was followed by cooling of the sample for 1 h at +4°C. Four milliliters of 0.034% $MgCl_2$ was added to the extract for each 1 g of tissue. The chloroform/methanol extracts were incubated overnight at +4°C to allow the organic (containing the extract of total lipids) and aqueous layers to separate completely. The upper (aqueous) layer was removed, and the lower (organic) layer was rinsed with chloroform/methanol (2:1 vol/vol), then placed into a glass tube. The total lipid fraction was obtained by evaporating the lower phase. The solvent was removed in a rotary evaporator under vacuum at +40°C. These extracts, representing the total lipids, were weighed, and results were noted for each fish. Total lipid contents were determined gravimetrically. After that, each extract was dissolved once again in 2 mL of chloroform/methanol (2:1 vol/vol). The resulting extract of total lipids was stored at +4°C until further analysis.

Analysis of lipid classes. Polar and neutral lipid fractions were separated from the total lipid extract by TLC. Chromatograms were developed on silica gel plates [Allurole Silica gel F_{254}; Merck, Darmstadt, Germany; 20 × 20 cm, 0.2 mm, using petroleum ether/diethyl ether (80:20, vol/vol)] up to 18 cm, so as to allow the separation of polar and neutral lipids. A small quantity of the sample was applied separately at the edge of the plate. That part of the chromatogram was cut off after development, and the bands were visualized by spraying with 50% sulfuric acid in ethanol followed by heating for 1 h at 180°C. Polar lipids remained at the start line, whereas neutral lipids moved along the plate. The position of the bands on the preparative part of the plate was determined by comparison with their position on the small, visualized part of the plate. Neutral lipids (TAG) were scraped off the plate together with the silica gel into tubes for methylation and further analysis. The same plate was put into the polar-lipid reagent (chloroform/methanol/ammonium hydroxide 65:35:5, by vol), up to the part where neutral lipids were scraped off. Polar lipid fractions (PE, PC, PI, PS) were visualized by iodine staining and scraped off the plate together with silica gel into tubes for methylation. Samples of polar and neutral lipid fractions, obtained as described, were used for FA analysis.

FA analysis. FA compositions of polar and neutral lipid fractions of fish muscle tissue samples were determined by GC of the corresponding methyl esters. FAME were obtained by acid methanolysis of lipid extracts by adding 0.86 mL of benzene and 1.00 mL of BF_3 in methanol to TAG and phospholipid fractions (8). A capillary gas chromatograph equipped with an FID was used. A nonpolar capillary column, HP Innowax cross-linked polyethyleneglycol (HP-5, 30 m × 0.32 mm; Agilent, Zagreb, Croatia) containing 5% diphenyl and 95% dimethylpolysiloxane, was used for analysis. The column temperature was programmed for a linear increase of 4°C/min from 150 to 210°C. The injector and detector temperatures were 250°C. Nitrogen was used as carrier gas. The analyses were performed twice. FAME were identified by comparing their retention times with those of commercial FAME standards (GLC 68B; Nu-Chek-Prep, Inc., Elysian, MN). The relative share of each identified FA for each polar and neutral lipid fraction was calculated automatically.

The degree of unsaturation, expressed as the unsaturation index according to Kates and Baxter (9), was calculated as follows: Δ/mol = [% monoene + 2 (% diene) + 3 (% triene) + 4 (% tetraene) + 5 (% pentaene) + 6 (% hexaene)]/100.

Statistical analysis. The results of FA composition were expressed as mean ± SD for each FA, representing a percentage of total FA. The Mann–Whitney U test was used as a nonparametric test for comparing the differences in FA composition for two independent groups of fish obtained in different seasons.

RESULTS AND DISCUSSION

Diplodus vulgaris is a very common fish in the Adriatic Sea. It is commercially important and exploited because it is appreciated in the Mediterranean type of diet. We also chose it as a target fish species because it does not migrate from its habitat once it settles there (10,11). In that way, differences in the influence of measured parameters such as water salinity, temperature, and pollution were to some extent avoided.

Body weights of *D. vulgaris* specimens analyzed in this study ranged from 200 to 400 g, with average lengths from 16 to 20 cm. Those values are within the limits reported in the literature (11). The total lipid content, expressed on a wet weight basis (%, w/w), amounted to 1.0 ± 0.4% in the winter period and 0.9 ± 0.3% in the summer period. According to the lipid content classification, this species is a low-fat fish (4). The water content of the samples was 77.8 ± 2.7% in the winter period and 76.6 ± 1.7% in the summer period. These results accord with our previously published results for the same fish species but from a different part of the Adriatic Sea (12).

Seasonal influence on the FA composition. The FA compositions of neutral and polar lipid fractions of *D. vulgaris* in winter and summer are shown in Tables 1 and 2, respectively. The relative concentrations of each FA are expressed as percentages of their total. The analyzed FA were grouped as saturated (SFA) and mono- and diunsaturated (MUFA + DUFA); tri-, tetra-, penta-, and hexaenoic FA were grouped as PUFA.

TABLE 1
FA Composition of Neutral (TAG) and Polar (PE, PC, PI/PS) Lipid Fractions of *Diplodus vulgaris* (expressed as percentage of total FA) in the Winter Period

	Percentage of total FA[a]			
FA component	TAG	PI/PS	PC	PE
14:0	5.9 ± 1.0	2.2 ± 1.9	1.4 ± 0.3	4.6 ± 4.3
16:0	21.9 ± 3.6	24.0 ± 8.5	44.7 ± 7.6	25.2 ± 7.0
16:1n-7	10.7 ± 1.7	1.8 ± 2.0	5.3 ± 0.5	4.3 ± 1.9
18:0	6.6 ± 0.9	17.2 ± 6.1	9.5 ± 4.0	20.7 ± 10.5
18:1n-9	32.8 ± 3.9	24.4 ± 15.0	19.9 ± 6.8	19.9 ± 5.8
18:2n-6	1.9 ± 0.7	1.8 ± 1.8	1.6 ± 0.8	3.9 ± 4.0
20:0	0.6 ± 0.4	0.2 ± 0.5	0.2 ± 0.3	0.8 ± 0.9
18:3n-3	2.6 ± 2.3	ND[b]	0.8 ± 0.9	1.2 ± 1.5
20:1n-9	2.5 ± 1.8	4.2 ± 3.9	0.9 ± 0.4	2.2 ± 1.4
22:0	0.3 ± 0.5	1.5 ± 2.0	1.5 ± 2.3	1.0 ± 2.5
20:4n-6	4.3 ± 1.8	7.4 ± 6.3	4.2 ± 4.8	6.7 ± 2.0
22:1n-11	1.0 ± 1.6	2.1 ± 2.6	1.3 ± 2.5	0.3 ± 0.7
20:5n-3	4.1 ± 0.9	0.9 ± 1.6	3.7 ± 2.7	2.7 ± 1.3
24:0	0.1 ± 0.2	1.0 ± 1.6	0.4 ± 0.4	0.4 ± 0.8
22:3n-3	2.2 ± 1.2	9.9 ± 9.1	1.1 ± 1.0	0.9 ± 0.8
22:6n-3	2.6 ± 1.8	1.3 ± 2.3	3.6 ± 2.7	5.3 ± 2.4
MUFA + DUFA	48.8 ± 4.9	34.3 ± 14.8	29.0 ± 7.0	30.6 ± 7.9
PUFA	15.7 ± 4.0	19.6 ± 12.3	13.3 ± 10.0	16.8 ± 7.9
Σ UFA	64.5 ± 3.3	53.8 ± 6.3	42.3 ± 9.1	47.4 ± 15.8
EPA + DHA	6.7 ± 2.6	2.2 ± 3.8	7.3 ± 4.5	8.0 ± 3.7
SFA	35.4 ± 3.3	46.2 ± 6.3	57.7 ± 9.1	52.7 ± 26.0
Unsaturation index[c]	1.18	1.08	0.93	1.13
n-3/n-6	1.85	1.32	1.59	0.95

[a]Values are mean ± SD.
[b]ND, not detected (<0.01%); MUFA, monounsaturated FA; DUFA, diunsaturated FA; UFA, unsaturated FA; SFA, saturated FA.
[c]The degree of unsaturation, expressed as the unsaturation index according to Kates and Baxter (9) (see text for complete formula).

TABLE 2
FA Composition of Neutral and Polar Lipid Fractions of *Diplodus vulgaris* (expressed as percentage of total FA) in the Summer Period

	Percentage of total FA[a]			
FA component	TAG	PI/PS	PC	PE
14:0	4.9 ± 1.1	1.5 ± 0.9	2.2 ± 1.7	0.7 ± 0.1
16:0	23.1 ± 2.4	29.6 ± 5.0	22.5 ± 8.9	39.6 ± 10.3
16:1n-7	7.3 ± 2.2	2.6 ± 2.9	3.6 ± 3.6	2.9 ± 0.9
18:0	11.4 ± 2.2	32.6 ± 16.3	36.5 ± 16.8	24.3 ± 20.4
18:1n-9	21.7 ± 2.5	5.9 ± 6.4	11.7 ± 8.5	15.0 ± 1.1
18:2n-6	2.8 ± 0.9	1.1 ± 1.0	1.5 ± 1.1	0.4 ± 0.3
20:0	0.3 ± 0.2	0.8 ± 1.1	0.5 ± 0.4	ND
18:3n-3	0.5 ± 0.6	0.8 ± 1.1	0.4 ± 0.5	0.1 ± 0.2
20:1n-9	1.8 ± 1.7	0.8 ± 1.4	0.5 ± 0.4	0.2 ± 0.2
22:0	0.7 ± 0.3	1.8 ± 1.4	1.1 ± 0.5	0.5 ± 0.7
20:4n-6	6.6 ± 2.7	7.7 ± 9.9	5.7 ± 4.2	4.9 ± 5.5
22:1n-11	ND	1.0 ± 1.4	Trace[b]	0.1 ± 0.1
20:5n-3	6.0 ± 2.0	1.7 ± 2.2	1.4 ± 1.6	2.1 ± 2.8
24:0	0.1 ± 0.2	1.9 ± 4.0	0.3 ± 0.2	0.1 ± 0.1
22:3n-3	5.1 ± 0.5	6.6 ± 3.3	7.5 ± 2.1	3.6 ± 2.3
22:6n-3	7.9 ± 1.0	3.7 ± 5.3	4.7 ± 2.9	5.5 ± 5.7
MUFA + DUFA	33.6 ± 2.5	11.3 ± 6.9	17.4 ± 11.6	18.5 ± 1.3
PUFA	26.0 ± 4.8	20.5 ± 13.7	19.7 ± 5.9	15.9 ± 13.4
Σ UFA	59.6 ± 4.3	31.8 ± 14.1	37.1 ± 11.8	34.4 ± 14.3
EPA + DHA	13.8 ± 2.8	5.4 ± 6.4	6.1 ± 3.8	7.3 ± 8.6
SFA	40.4 ± 4.3	68.2 ± 14.1	64.5 ± 10.8	65.3 ± 14.1
Unsaturation index[c]	1.56	0.96	1.01	0.93
n-3/n-6	2.07	1.45	1.94	2.13

[a]Values are mean ± SD.
[b]Trace, <0.1%; for other abbreviations see Table 1.
[c]The degree of unsaturation, expressed as the unsaturation index according to Kates and Baxter (9) (see text for complete formula).

We identified 16 different FA in the fish muscle tissue samples analyzed. The major constituents of total FA in both seasons were saturates: palmitic (16:0) and stearic acids (18:0); monoenes: oleic (18:1n-9) and palmitoleic acids (16:1); and polyunsaturates: arachidonic acid (20:4n-6), EPA (20:5n-3), and DHA (22:6n-3). The major FA identified in our study were 16:0, 18:0, and 18:1n-9; their amounts and ratios differed significantly between the two seasons and between lipid fractions. Donato *et al.* (13) made a similar observation for this fish species in other areas of catch in the Adriatic Sea. Hazra *et al.* (14) reported that saturates and monoenes constitute 60–70% of total FA in puffer fish. The FA profile of *D. vulgaris* generally fits into the typical pattern for fresh-caught fish, where oleic acid is usually the major constituent, with seasonal variation (15). A statistically significant difference ($P < 0.0001$) in oleic acid (18:1n-9) content was found between summer and winter. This FA showed the greatest seasonal variation in this study, followed by 18:0 and 16:0. Values for 18:0 in TAG and PC were found to be statistically different ($P < 0.0001$) during the two periods. The content of 18:0 was considerably higher in summer, when the relative ratio of 18:0 was almost two times higher for TAG and almost four times higher for PC than in the winter period. No statistically significant seasonal variation was detected in the relative ratio of 16:0 in TAG and PI/PS, but it was noticeable in PC and PE ($P < 0.05$). Values for 16:0 were twice as high in winter in PC. In contrast, for PE the relative ratio of 16:0 was much higher in the summer. The content of 18:1n-9 significantly decreased from winter to summer ($P < 0.05$). This is in agreement with the results of Donato *et al.* (13) for *D. vulgaris* originating from the Adriatic Sea.

The concentrations of n-3 PUFA, EPA, and DHA are significant for their biomedical importance. A statistically significant difference in TAG ($P < 0.05$) was found in the EPA and DHA content, with greater amounts in the summer. No such difference was found in polar lipid fractions. EPA + DHA values were twice as high in the summer period in TAG and PI/PS. Appreciable quantities of 20:4n-6 and 22:3n-3 were also found in all the lipid fractions, with statistically significant seasonal differences ($P < 0.0001$) in TAG, PC, and PE for 22:3n-3. Seasonal variation in the 20:4n-6 content was significant only in TAG ($P < 0.05$). Generally, MUFA + DUFA values were significantly higher in winter. On the other hand, PUFA values were higher in summer, especially in TAG. SFA values were also higher in summer. The diminution of the MUFA content in the summer was clearly accompanied by an increase in PUFA content. This is in agreement with the observations of Donato *et al.* (13). The TAG serve as a store for SFA for energy purposes, and they also may be a temporary PUFA reservoir (16), which could be forwarded to the structural lipids or directed to specific metabolic pathways. Statistically significant seasonal differences ($P < 0.05$ and $P < 0.0001$) were most conspicuous only in TAG (neutral lipid fractions) for all the detected FA except 16:0, 20:0, 18:3n-3, 20:1n-9, 22:1n-11, and 24:0. Pazos *et al.* (17) reported a similar observation. On the other hand, statistically significant differences ($P < 0.05$ and $P < 0.0001$) in polar lipid fractions (PI/PS, PC, and PE) were found to be less noticeable, especially in PI/PS, where statistically significant seasonal variation was found only for 18:1n-9 ($P < 0.0001$). Statistically significant seasonal variations ($P < 0.05$ and $P < 0.0001$) from winter to summer in PC were found in the relative ratios of these FA: 16:0, 18:0, 20:0, 20:1n-9, 22:1n-11, and 22:3n-3. When analyzing for statistically significant differences in PE, they were found in the relative ratios of 14:0, 16:0, 18:1n-9, 18:2n-6, 20:1n-9, and 22:3n-3.

The degree of unsaturation, expressed as the unsaturation index, also differed between neutral and polar lipid fractions. It was highest in TAG during the summer. When compared with the results of our previous research (12), the results of this study showed higher values for the PI/PS and PC unsaturation indexes, which are in agreement with our previously published results for the TAG and PE unsaturation indexes. In recent years, n-3 PUFA have been proved to have greater beneficial influence in the amelioration of heart and cardiovascular disorders than n-6 PUFA. Recent emphases on n-3 PUFA over n-6 PUFA propose that the n-3/n-6 ratio could be applied as a biomedical index (1). Therefore, the n-3/n-6 ratio is a biomedical marker for fish lipids. n-3/n-6 ratios were calculated for all the lipid fractions in analyzed fish muscle tissue samples. FA in *D. vulgaris* muscle tissue lipids have an n-3/n-6 ratio between 1 and 2, which is quite good. But it must be emphasized that all the ratios were higher in summer.

Our study indicates that *D. vulgaris* is a good source of natural n-3 PUFA and would therefore be suitable for inclusion in highly unsaturated low-fat diets. The results of our study are in agreement with other published results for teleost fish species originating from the Mediterranean and Adriatic Seas (12,13,18,19). Seasonal variations of FA composition have previously been studied for different fish species (4,13,14,17,19). An inverse relationship between water temperature and the amount of PUFA in tissue lipids of fish and invertebrates has been shown (20). Seasonal variation of n-3 PUFA seems to be linked to the diet as well as the reproductive cycle, which was also reported by Donato *et al.* (13) for *D. vulgaris* from the Adriatic Sea. In this study, the FA composition of *D. vulgaris* showed a significant variation from winter to summer. The seasonal variations in *D. vulgaris* lipids reflected fluctuations mainly in TG. But it must also be emphasized that the reproductive cycle of *D. vulgaris* correlates with those seasons, because previtellogenesis occurs in winter and vitellogenesis occurs in summer (10,11,13). We can conclude that, although the FA composition of fish is complex and depends on many factors, it clearly shows a seasonal pattern.

ACKNOWLEDGMENT

This research was supported by a grant from the Ministry of Scientific Research and Technology, Republic of Croatia (project no. 006210).

REFERENCES

1. Hu, F.B., L. Bronner, W.C. Willet, M.J. Stampfer, K.M. Rexrode, C.M. Albert, D. Hunter, and J.E. Manson, Fish and Omega-3 Fatty Acid Intake and Risk of Coronary Heart Disease in Women, *JAMA 287*:1815–1821 (2002).
2. Kinsella, J.E., B. Lokesh, and R.A. Stone, Dietary n-3 Polyunsaturated Fatty Acids and Amelioration of Cardiovascular Disease: Possible Mechanisms, *Am. J. Clin. Nutr. 52*:1–28 (1990).
3. Mori, T.A., and L.J. Beilin, Long-chain Omega 3 Fatty Acids, Blood Lipids and Cardiovascular Risk Reduction, *Curr. Opin. Lipidol. 12:*11–17 (2001).
4. Ackman, R.G., Fatty Acids, in *Marine Biogenic Lipids, Fats and Oils,* edited by R.G. Ackman, CRC Press, Boca Raton, 1989, pp. 145–178.
5. Windsor, M., and S. Barlow, *Introduction to Fishery By-products*, Fishing New Books, Farnham, United Kingdom, 1981, pp. 1–189.
6. Ackman, R.G., Nutritional Composition of Fats in Seafoods, *Prog. Food Nutr. Sci. 13*:161–289 (1989).
7. Folch, J., M. Lees, and G.H. Sloane Stanley, A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem. 226*:497–509 (1957).
8. Metcalfe, L.D., and A.A. Schmitz, The Rapid Preparation of Fatty Acid Esters for Gas Chromatographic Analysis, *Anal. Chem. 33*:363–364 (1961).
9. Kates, M., and R.M. Baxter, Lipid Composition of Mesophilic and Psychrophilic Yeasts (*Candida* species) as Influenced by Environmental Temperature, *Can. J. Biochem. Physiol. 40*:1212–1227 (1962).
10. Jug-Dujakovic, J., and B. Glamuzina, Preliminary Studies of Reproduction and Early Life History of *Diplodus vulgaris* (E. Geoffroy Saint-Hilarie 1817) in Captivity, *Aquaculture 69*:367–377 (1988).
11. Jardas, I., *Jadranska ihtiofauna,* Školska knjiga, Zagreb 1996, pp. 275–276.
12. Varljen, J., S. Sulic, J. Brmalj, L. Baticic, V. Obersnel, and M. Kapovic, Lipid Classes and Fatty Acid Composition of *Diplodus vulgaris* and *Conger conger* Originating from the Adriatic Sea, *Food Technol. Biotechnol. 41*:149–156 (2003).
13. Donato, A., G. Dugo, A. Mauceri, and A. Verzera, Changes in Fatty Acids Composition in *Diplodus vulgaris* Liver in Relation to Sexual Maturation, *Rio. Ital. Sostanze Grasse 59*:349–353 (1984).
14. Hazra, A.K., S. Ghosh, S. Banerjee, and B. Mukherjee, Studies on Lipid and Fatty Acid Compositions of Puffer Livers from Indian Coastal Waters with Seasonal Variation, *J. Am. Oil Chem. Soc. 75*:1673–1678 (1998).
15. Kamler, E., B. Krasicka, and S. Rakusa-Suszczewski, Comparison of Lipid Content and Fatty Acid Composition in Muscle and Liver of Two Notothenioid Fishes from Admiralty Bay (Antarctica): An Eco-physiological Perspective, *Polar Biol. 24*:735–743 (2001).
16. Napolitano, G.E., B.A. MacDonald, R.J. Thompson, and R.G. Ackman, Fatty Acid Components of Larval *Ostrea edulis* (L.): Importance of Triacylglycerols as a Fatty Acid Reserve, *Comp. Biochem. Physiol. 90B*:875–883 (1988).
17. Pazos, A.J., C. Ruiz, O. Garcia-Martin, M. Abad, and J. Sanchez, Seasonal Variations of the Lipid Content and Fatty Acid Composition of *Crassostrea gigas* Cultured in El Grove, Galicia, N.W. Spain, *Ibid. 114B*:171–179 (1996).
18. Passi, S., S. Cataudella, P. Di Marco, F. De Simone, and L. Rastrelli, Fatty Acid Composition and Antioxidant Levels in Muscle Tissue of Different Mediterranean Marine Species of Fish and Shellfish, *J. Agric. Food Chem. 50*:7314–7322 (2002).
19. Mayzaud P., P. Virtue, and E. Albessard, Seasonal Variations in the Lipid and Fatty Acid Composition of the Euphausiid *Meganyctiphanes norvegica* from the Ligurian Sea, *Mar. Ecol. Prog. Ser. 186*:199–210 (1999).
20. Hazel, J.R., Influence of Thermal Acclimation on Membrane Lipid Composition of Rainbow Trout Liver, *Am. J. Physiol. 236*:91–101 (1979).

[Received August 11, 2003; accepted July 1, 2004]