Variation in fat content and fatty-acid composition of the Baltic herring *Clupea harengus membras*

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The fat content and fatty-acid profiles of herring, *Clupea harengus membras*, from the southern Baltic Sea varied depending on when (fishing season) and where (fishing grounds) the fish were caught as well as on their size and sex. The fat, protein and dry matter content and the fatty-acid profiles were assayed in *C. h. membras* muscle tissue. The changes observed in fatty-acid profiles were determined by factors such as specimen mass and fat content, which, in turn, depended on fishing season. This is explained by dietary differences between juvenile and older fish. Gonad maturation and spawning in the latter are also factors. The study results provide confirmation of the hypothesis that polyunsaturated fatty acids (PUFA), in particular docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), play vital roles in the sexual maturation of *C. h. membras*.

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Key words: Baltic Sea; biological characteristics; DHA; EPA.

**INTRODUCTION**

In recent years, increasing attention has been focused on the significance of polyunsaturated fatty acids (PUFA) in human and animal nutrition, particularly eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and, to a lesser degree, docosapentaenoic acid (DPA). These long-chain, polyunsaturated fatty acids (LC-PUFA), acids comprising ≥20 carbon atoms and at least three double bonds (Graham *et al.*, 2007), play roles in regulating biochemical and physiological processes, because they are the major components of cellular membranes. That is why they are known as the ‘fats of life’ (Pond, 1998).

LC-PUFA can be classed according to the position of the first double bond in n–3 and n–6 families. EPA, DHA and DPA belong to the n–3 family of fatty acids. The most significant amounts of these acids are contained in marine fish oils. According to many researchers (Leskanich & Noble, 1997; Kolanowski, 2000), the prophylactic effect of fatty acids is determined by, among other factors, the ratio of n–6 to n–3 acids, which should be 6–4:1 in human nutrition. This should include an
average daily intake of c. 8 g mix of essential unsaturated fatty acids (EUFA) (linoleic acid: C18:2n6, α-linolenic acid: C18:3n3, γ-linolenic acid: C18:3n6, arachidonic acid: C20:4n6, eicosapentaenoic acid: C20:5n3 and docosahexaenoic acid: C22:5n3) (Holman, 1998). These acids are essential since they cannot be synthesized in the human body and have to be supplied by food. Acids C20 and C22 are derived through the bioconversion of C18:2n6 and C18:3n3 through subsequent stages of elongase and desaturase (Graham et al., 2007). Maintaining the proper ratio of n−3 and n−6 acids in the diet can contribute to general improvements in health and reduced risks of cancer while also having a beneficial effect on the immune system. Nevertheless, according to other researchers (Kris-Etherton et al., 2002; Block & Pearson, 2006), the optimal ratio of these two groups of acids has yet to be established.

High diversity in fatty-acid profiles, especially with regard to the content of the beneficial EPA and DHA, is noted among fish species, including those that inhabit the Baltic Sea (Aro et al., 2000; Aidos et al., 2002). The proximate composition of fish species changes depending on the season, maturity stage and fishing ground (Aidos et al., 2002). The diet varies among seasons, which induces a seasonal variation in fat content, and then in the concentrations of EPA and DHA. Research conducted by scientists from Spain (Satue & Lopez, 1996), Uruguay (Méndez & González, 1997), Finland (Aro et al., 2000), Japan (Shirai et al., 2002), Brazil (Luzia et al., 2003) Turkey (Gokce et al., 2004), Russia (Gladyshev et al., 2007), France (Le Nechett et al., 2007), Tunisia (Mnari et al., 2007) and Greece (Zlatanos & Laskaridis, 2007) on different fish species [rainbow trout *Oncorhynchus mykiss* (Walbaum), Argentine hake *Merluccius hubbsi* (Marini), *Clupea harengus membras* L.; sardines *Sardinops melanostictus* (Temmincket & Schlegel), tilapia *Oreochromis* spp., sole *Solea solea* (L.), brown trout *Salmo trutta* L., herring *Clupea harengus pallasi* (Valenciennes), rock sole *Lepidopsetta bilineata* (Ayres), cod *Gadus morhua marisalbi* (Derjugin), ray, *Himantura bleekeri* (Blyth), sea bream *Sparus aurata* L., sardine *Sardina pilchardus* (Walbaum), anchovy *Engraulis encrasicholus* L. and picarel *Spicara smaris* (L.)] confirms correlations between fatty-acid composition and fishing season, fish food composition, fish size and the processing technology used to prepare them for consumption. For example, studies by Satue & Lopez (1996) indicated that male *O. mykiss* individuals were characterized by higher contents of PUFA than were female individuals, which, according to the authors, was linked to the use of EPA and DHA by females for egg formation. In turn, Shirai et al. (2002) reported seasonal changes in the fatty-acid profiles of *S. melanostictus* depending on the composition of these acids in the plankton on the fishing grounds. For example, they demonstrated that the profile of the fatty acids isolated in *S. melanostictus* was similar to those of the plankton from the same period. Studies by Gladyshev et al. (2007) demonstrated, however, that thermal processing (boiling and frying) did not cause a significant decrease in the contents of either EPA or DHA in comparison with raw fish.

*Clupea h. membras* is a pelagic fish which, as it goes through its natural biological cycle, has varied contents of fat and other components (Oterhals, 1995; Aro et al., 2000). The food industry exploits this in the production of marinated, canned, frozen, smoked and salted fish since fat content differs in various seasons, and because fatty fish products are recommended by nutritional authorities as a source of beneficial LC-PUFA (Aidos et al., 2002). The majority of papers published to date regarding seasonal changes in fat content and fatty-acid profile in Atlantic herring *Clupea*
FATTY-ACID PROFILES IN *C. HARENGUS MEMBRAS*  

**harengus** L. have focused on fish inhabiting the North Sea, Norwegian waters and the eastern part of the Baltic Sea. The current study concerns *C. h. membras* caught in the southern Baltic Sea (Polish catch area).

**MATERIALS AND METHODS**

**DESCRIPTION OF FISH**

*Clupea h. membras* inhabiting the Baltic Sea is characterized by substantial intraspecific variation. Fish of this species, even those that occur within close proximity, often differ significantly regarding anatomical and physiological structures (e.g. number of vertebrae, body proportions, growth rate and degree of sexual development) and feeding migration routes. From a taxonomic viewpoint, *C. h. membras* is a subspecies of *C. harengus*. In the Baltic Sea, there are two basic reproductive populations of *C. h. membras*: spring and autumn spawning. Since the mid 1960s, the spring spawning *C. h. membras* has been the numerically dominant group. It spawns from March to May, and during this period up until the end of June it is spent. For at least the past decade, the number of autumn *C. h. membras* has changed only slightly and has not exceeded 10% of the total number of the species (Wyszyński, 1997). In the Polish catch area, the spring *C. h. membras* spawning grounds are predominant in the inshore zone. Since the research samples came from this area, the results of the study were interpreted with reference to the spring spawning *C. h. membras*.

**MATERIAL ANALYSED**

The samples were collected in 2007 and 2008 either during scheduled cruises of the R.V. Baltica or they were obtained from fishing cutters operating in the Polish catch area (Fig. 1). During the study period, 100 *C. h. membras* samples were collected, and the sampling site and date were recorded for each sample. The size of the specimens [wet mass (*M*$_{S}$) and total length (*L*$_{T}$) class] was measured. Individuals were weighed to the nearest 0·1 g and measured to the nearest 0·5 cm. The samples were sealed in plastic bags and frozen on board the ship at a temperature of −18°C, or, if purchased from fishermen, fresh samples were taken directly to the laboratory.

In the laboratory, the sex of the fish was determined visually and composite samples consisting of 30 specimens were prepared. The fish were pooled into samples according to

![](https://example.com/image.png)

**Fig. 1.** Fishing grounds where samples were caught in ICES sub-divisions 24, 25 and 26 in the Baltic Sea.

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injection; split ratio: 100:1; injection volume: 2 μl in the heating block for 15 min and then cooled to room temperature. An aliquot of 1.0 ml of the sample was injected into the chromatograph. The chromatographic analysis of the fatty acids was performed after they had been passed through the appropriate methyl esters (FAME) (EN ISO standard 5509:2000). The percentage contents of MDF, PC and FC were determined in relation to the M of the muscle tissues.

Fatty acids were determined for the total lipid fraction; 5 g of freeze-dried samples were extracted with 80 ml of a hexane:acetone mixture (4:1 v/v) in a Soxtec Avanti apparatus (www.foss.dk) for 4 h. The solvents were evaporated carefully in a rotary evaporator under reduced pressure and a stream of nitrogen. The chromatographic analysis of the fatty acids was performed after they had been passed through the appropriate methyl esters (FAME) (EN ISO standard 5509:2000; www.iso.org). A 0.2 g sample of the extracted lipid was dissolved in 1.6 ml of 2 M methanolic potassium hydroxide solution and shaken vigorously. The solution was heated for 15 min in a heating block, and then cooled to room temperature. Subsequently, 3.2 ml of 4% methanolic solution of hydrochloric acid was added. The samples were reheated in the heating block for 15 min and then cooled to room temperature. An aliquot of 1.6 ml of isooctane was added and the solution was vortexed and adjusted to a volume of 10 ml with a saturated solution of sodium chloride. Anhydrous sodium sulphate was added to dry extracts. The resultant solution of FAME on the top layer was diluted with methanol in proportion 1:4 v/v and was subjected to the final determination (EN ISO standard 5509:2000). Extracts were analysed with an Agilent 6890N GC gas chromatograph (www.agilent.com) equipped with a flame-ionization detector (FID). The column used was a Supelco SP 2560 (100 m length and 0.25 mm internal diameter; www.sigmaaldrich.com). Chromatography conditions were split injection; split ratio: 100:1; injection volume: 2 μl; carrier gas flowing at 1.1 ml min⁻¹; helium; injection port temperature: 250°C; detector temperature: 260°C; oven temperature: initial oven temperature 140°C held for 2 min then increased to 225°C at a rate of 2°C min⁻¹ and held for 10 min followed by an increase to 240°C at a rate of 4°C min⁻¹ and held for 10 min. Identification and quantification were performed based on retention times and areas of peaks in the standard mixture (37 FAME Mix, C18:4 n−3, Supelco and C22:5 n−3, Supelco), using Agilent Technologies software [Chemstation; Rev A 10.02 (1757)].

The content of components was expressed as percentages by the mass of the methyl esters (Pm). Correction factors (Ki) were used to convert the percentages of peak areas into mass percentages of the components. Ki were determined with a chromatogram derived from the analysis of a standard mixture carried out under operating conditions identical to those used for the samples according to the following formula (EU, 1991): 

\[ K_i = \left( \frac{m_i \sum A_i}{m \sum A} \right) \left( \frac{A_i \times \sum m}{\sum m} \right)^{-1} \]

where \( m_i \) is the mass of component \( i \) in the standard mixture, \( \sum m \) is the total of the masses of the various components of the standard mixture, \( A_i \) is the area under the peak corresponding to component \( i \) and \( \sum A \) is the sum of the areas under all the peaks.

The content of fatty acids \( C_{fa} \) expressed in mg g⁻¹ tissue was calculated using a conversion factor of 0.956 (Méndez et al., 1996; Vasilopoulou et al., 2003) and the total lipid content of fish according to the following formula: 

\[ C_{fa} = \left( 10^4 P_{fa} 0.956 F_C \right) 10^{-4} \]

Certified reference material NIST 8415 (whole egg powder) was used to validate the method. The laboratory has participated in intercalibration trials organized by FAPAS (FAPAS 1450; www.fapas.com) and has achieved positive results [the z-scores for saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA were 0.8, 0.0 and 0.3, respectively].

SAMPLE ANALYSIS

The proximate composition was determined at the Sea Fisheries Institute’s accredited laboratory (Accreditation Certificate no. AB 017 awarded by the Polish Centre of Accreditation) with the following AOAC (1990) procedures: dry matter (MDF, %) was determined by drying samples in an oven at 105°C for 8 h; overall protein (PC, %) was determined with the Kjeldahl method (AOAC, 1990) after acid digestion using a conversion factor of 6.25; fat content (FC, %) was determined gravimetrically after Soxhlet extraction with petroleum ether (AOAC, 1990). The percentage contents of SFA, MUFA and PUFA were 0.956 (Mendez et al., 1996; Vasilopoulou et al., 2003) and the total lipid content of muscle tissues.
DATA ANALYSIS

Statistical analyses were conducted with the STAT statistical software package (Statistica, Version 8.0; www.statsoft.com). The data were log_{10} transformed, and the significance level was \( P < 0.05 \). The homogeneity of variances was examined using Levene’s test. \( t \)-tests with the estimation of variance (Cohran–Cox test) were applied to determine whether the differences in \( F_C \), \( M_D \), \( P_C \) and EPA + DHA among individuals caught in different seasons, at different fishing grounds, in different \( LT \) classes or between the sexes were statistically significant. ANOVA (Scheffe test) was applied to verify whether there are differences in the \( n-3:n-6 \) ratio among individuals in different \( LT \) classes. Pearson’s correlation analyses were applied to determine the linear correlation between EPA, DHA, EPA + DHA and \( M_S \) and multiple regression analyses were used to analyse the simultaneous influence of sex, \( M_S \) and \( F_C \) on EPA and DHA. Values in this paper are mean ± s.d.

The comparative statistical tests considered: (1) fishing ground, Baltic Sea ICES subdivisions 24, 25 and 26; (2) fishing period, spring to summer (February to June) and summer to autumn (July to November); (3) fish sex (male or female) and 4) \( LT \), <20 cm (class 1), 20–24 cm (class 2) and >24 cm (class 3).

RESULTS

PROXIMATE COMPOSITION

The \( F_C \) in the muscles of fish from different \( LT \) classes (1–3) did not differ significantly (Table I).

The average \( F_C \) in muscle tissue of fish sampled during the summer to autumn period (5.3 ± 1.8%) was statistically significantly higher (\( P < 0.001 \)) than in fish sampled in the spring to summer period (2.7 ± 1.1%) (Table II). The average \( F_C \) in the analysed C. h. membras ranged from 2.5 ± 1.0% in April (spring C. h. membras

Table I. Proximate composition [dry matter (\( M_D \)), protein content (\( P_C \)), fat content (\( F_C \)) and fish wet mass (\( M_S \))] of Clupea harengus membras muscle tissue (mean ± s.d.) depending on total; length classes (\( LT \)): 1, <20 cm; 2, 20–24 cm; 3 >24 cm. Different superscript lowercase letters in columns indicate means are significantly different (\( P \leq 0.05 \))

<table>
<thead>
<tr>
<th>Fish length class</th>
<th>( n )</th>
<th>( M_D ) (%)</th>
<th>( P_C ) (%)</th>
<th>( F_C ) (%)</th>
<th>( M_S ) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>35</td>
<td>23.4 ± 2.0</td>
<td>18.1 ± 0.5</td>
<td>4.1 ± 2.1</td>
<td>47.6 ± 8.9a</td>
</tr>
<tr>
<td>Class 2</td>
<td>37</td>
<td>23.3 ± 2.0</td>
<td>17.9 ± 0.5</td>
<td>4.3 ± 2.0</td>
<td>74.3 ± 11.2b</td>
</tr>
<tr>
<td>Class 3</td>
<td>28</td>
<td>22.6 ± 2.0</td>
<td>17.8 ± 0.4</td>
<td>3.8 ± 1.8</td>
<td>113.6 ± 13.7c</td>
</tr>
</tbody>
</table>

\( n \), number of fish.

Table II. Seasonal changes (July to November and February to June) in proximate composition (see Table I) of Clupea harengus membras muscle tissue (mean ± s.d.). Different superscript lowercase letters in columns indicate means are significantly different (\( P \leq 0.05 \))

<table>
<thead>
<tr>
<th>Fishing period</th>
<th>( n )</th>
<th>( M_D ) (%)</th>
<th>( P_C ) (%)</th>
<th>( F_C ) (%)</th>
<th>( M_S ) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July to November</td>
<td>52</td>
<td>24.4 ± 1.8a</td>
<td>18.0 ± 0.3a</td>
<td>5.3 ± 1.8a</td>
<td>69.5 ± 21.2a</td>
</tr>
<tr>
<td>February to June</td>
<td>48</td>
<td>21.8 ± 1.2b</td>
<td>17.9 ± 0.6a</td>
<td>2.7 ± 1.1b</td>
<td>82.9 ± 33.5b</td>
</tr>
</tbody>
</table>

\( n \), number of fish.

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spawning season) to 8.0 ± 1.6% in October (end of intense feeding period of spring *C. h. membras*). The correlation between *F*<sub>C</sub> in male and female specimens according to fishing season are shown in Fig. 2. The statistical analysis revealed significant *F*<sub>C</sub> differences between the muscle tissues of fish collected during summer to autumn and spring to summer seasons for both female (*P* < 0.05) and male (*P* < 0.001) *C. h. membras*.

It was noted that a statistically significant higher *F*<sub>C</sub> was observed in males (4.7 ± 2.2%), compared to females (3.9 ± 2.0%), (*P* < 0.05) (Table III). When sampling season was taken into account as an additional factor, the difference in *F*<sub>C</sub> between the muscle tissues of both sexes was more pronounced in the spring to autumn season (Fig. 2). Differences in *F*<sub>C</sub> in fish caught in various basins of the Polish catch area (ICES sub-divisions 24, 25 and 26) were investigated. No statistically significant differences were found in sub-division 24 or 25, whereas the *F*<sub>C</sub> from sub-division 26 was characterized by a substantially higher value than from sub-division 25 (*P* < 0.01) (Table IV).

A statistically significant lower level in *P*<sub>C</sub> was observed in muscle tissue of specimens >24 cm compared to smaller fish (classes 1–3, *P* < 0.01; classes 2–3, *P* > 0.05) (Table I).

### FATTY-ACID PROFILE

The dominant SFA was palmitic acid C16:0 (20.1 ± 1.2%), the dominant MUFA was oleic acid C18:1n−9c (22.2 ± 4.7%) and among the PUFA it was docosahexaenoic

**Table III.** Proximate composition (see Table I) of *Clupea harengus membras* muscle tissue (mean ± s.d.) depending on sex. Different superscript lowercase letters in columns indicate means are significantly different (*P* ≤ 0.05)

<table>
<thead>
<tr>
<th>Sex</th>
<th>n</th>
<th><em>M</em>&lt;sub&gt;D&lt;/sub&gt; (%)</th>
<th><em>P</em>&lt;sub&gt;C&lt;/sub&gt; (%)</th>
<th><em>F</em>&lt;sub&gt;C&lt;/sub&gt; (%)</th>
<th><em>M</em>&lt;sub&gt;S&lt;/sub&gt; (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>33</td>
<td>23.1 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.6 ± 30.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>23.8 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.0 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7 ± 2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.5 ± 27.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*n*, number of fish.
TABLE IV. Proximate composition (see Table I) of *Clupea harengus membras* muscle tissue (mean ± s.d.) by ICES fishing areas (see Fig. 1). Different superscript lowercase letters in columns indicate means are significantly different (*P* ≤ 0·05)

<table>
<thead>
<tr>
<th>ICES areas</th>
<th>n</th>
<th> <em>M</em>D (%)</th>
<th> <em>P</em>C (%)</th>
<th> <em>F</em>C (%)</th>
<th> <em>M</em>S (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>31</td>
<td>23·3 ± 1·9a</td>
<td>18·1 ± 0·4a</td>
<td>3·9 ± 2·1a</td>
<td>75·1 ± 29·7a</td>
</tr>
<tr>
<td>25</td>
<td>38</td>
<td>22·4 ± 1·8a</td>
<td>17·9 ± 0·5a</td>
<td>3·4 ± 1·5a</td>
<td>82·1 ± 28·8a</td>
</tr>
<tr>
<td>26</td>
<td>31</td>
<td>23·9 ± 2·1b</td>
<td>17·9 ± 0·3a</td>
<td>5·0 ± 2·1b</td>
<td>69·3 ± 25·9a</td>
</tr>
</tbody>
</table>

*n*, number of fish.

acid C22:6n−3 (20·2 ± 4·9%). The current study indicated that there were major changes both in the percentage composition of the fatty acids in individual months (Fig. 3) and in the concentration of fatty acids (mg g⁻¹ tissue) (Fig. 4).

In percentage terms, PUFA predominated in the lipids extracted from the muscle tissue of the fish tested in the current study, and ranged from 35·9 ± 5·9 to 48·7 ± 2·8% (Fig. 3). Taking into account the concentration of fatty acids (Fig. 4), the greatest variations were noted in the PUFA content, which reached the maximum level near the end of intense feeding period in October at 28·8 ± 5·5 mg g⁻¹ tissue, while in the postspawning period in April (when the fish are spent or when they begin to rebuild their energy resources) this decreased to 8·2 ± 2·7 mg g⁻¹ tissue. Less pronounced changes were observed in the content of SFA, which ranged from 7·1 ± 3·0 mg g⁻¹ tissue in the postspawning period to 23·6 ± 5·4 mg g⁻¹ tissue towards the end of the intense feeding period. Changes were also less pronounced

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**Fig. 3.** Seasonal changes of saturated fatty acids (SFA; □), monounsaturated fatty acids (MUFA; ■) and polyunsaturated fatty acids (PUFA; ▪) percentage content in the total lipid fraction extracted from muscle tissues of *Clupea harengus membras* from the southern Baltic Sea. Value are means ± 95% CI.
Fig. 4. Seasonal changes of saturated fatty acids (SFA; □), monounsaturated fatty acids (MUFA; ■) and polyunsaturated fatty acids (PUFA; □) concentrations in the muscle tissue of *Clupea harengus membras* from the southern Baltic Sea. Values are means ± 95% CI.

in the content of MUFA, which ranged from 8.1 ± 2.7 mg g⁻¹ tissue in April to 24.0 ± 5.5 mg g⁻¹ tissue in October.

The seasonal changes in EPA and DHA content in *C. harengus membras* muscle tissue are shown in Fig. 5. The content of DHA was lowest in the postspawning

Fig. 5. Seasonal changes of eicosapentaenoic acid (EPA; □) and docosapentaenoic acid (DHA; ■) concentrations in the muscle tissue of *Clupea harengus membras* from the southern Baltic Sea. Values are means ± 95% CI.
period (4.2 ± 1.5 mg g⁻¹ tissue) and increased during the intensive feeding period to concentrations of 15.1 ± 2.5 mg g⁻¹ tissue. In contrast, EPA content varied within a narrower range from 1.6 ± 0.5 to 4.7 ± 0.8 mg g⁻¹ tissue.

The relationship between the EPA and DHA (%) content and muscle mass was also examined. Statistical analysis indicated that the EPA, DHA and the sum of EPA and DHA in the total lipid fraction was negatively correlated with mean muscle mass ($r = -0.57, -0.70$ and $-0.75$, respectively, $P < 0.001$) (Fig. 6). The results indicate that the content of EPA and DHA is higher in specimens of lower mass. A similar correlation was observed when total length was considered. The analysis confirmed that shorter specimens contained significantly higher contents of EPA (classes 1–3, $P < 0.01$; classes 1–2, $P < 0.01$; classes 2–3, $P > 0.05$) and DHA (classes 1–3, $P < 0.001$; classes 1–2 $P < 0.01$; classes 2–3 $P < 0.01$) than did longer ones.

A correlation between fish condition and the content of EPA and DHA in the total lipid fraction was also investigated. No significant linear correlations were found ($P > 0.05$).

The influence of sex on the content of EPA and DHA (%) in the total lipid fraction taking into consideration total length and the sampling season is shown in Fig. 7. No significant differences were found between males and females.

Multiple regression was used to analyse the simultaneous influence of sex, muscle mass and fish condition on the content of EPA and DHA in the total lipid fraction. Only the coefficient of muscle mass was statistically significant for EPA, while for DHA the coefficients of the two variables of muscle mass and fish condition were statistically significant. The standardized regression coefficient β, however, indicated that the variable muscle mass was the one that contributed most to the prediction of DHA (%). The correlation coefficient obtained for the equation with two variables ($r = -0.73, P < 0.001$) was slightly better than for the equation with only one variable muscle mass ($r = -0.70$).

The n−3:n−6 fatty-acid ratio depended on the fishing season and the L class (Fig. 8). The differences in the n−3:n−6 fatty acids ratio between the fish sampled in the summer to autumn period and the spring to summer period were slight, while variation in this ratio was greater between the individual L classes. In both seasons, the n−3:n−6 ratio in fish from class 1 (<20 cm) was statistically significantly higher.
Fig. 7. Comparison of eicosapentaenoic acid (EPA) + docosapentaenoic acid (DHA) content in the total lipid fraction extracted from the muscle tissue of *Clupea harengus membras* from the southern Baltic Sea between male (M) and female (F) specimens according to season (a) July to November and (b) February to June and total length (*L*<sub>T</sub>) classes (1, <20 cm; 2, 20–24 cm; 3, >24 cm). Values are mean ± 95% c.i. Different lowercase letters indicate means are significantly different (*P* < 0·05).

than that measured in fish from class 3 (>24 cm) (season spring to summer *P* < 0·01; season summer to autumn *P* < 0·05).

**DISCUSSION**

**PROXIMATE COMPOSITION**

Slight fluctuations of *P*<sub>C</sub> were observed during the year. The *P*<sub>C</sub> measured in the current study was comparable to that reported by Aidos *et al.* (2002) in *C. harengus* fillets from the North Sea (from 16·0 to 18·9%).

The maximal mean ± s.d. *F*<sub>C</sub> observed in the current study in October (8·0 ± 1·6%) and in *C. h. membras* from the eastern part of the Baltic Sea (10%; Aro *et al.*, 2000) were lower in comparison to a maximal *F*<sub>C</sub> measured in *C. harengus* from the North Sea (19%; Aidos *et al.*, 2002) or in Norwegian *C. harengus* (17·5 ± 0·4%; Hamre *et al.*, 2003). Such differences are probably associated with the prevailing feeding conditions in these regions (food availability and caloric value of this food).

Seasonal variation in the average *F*<sub>C</sub> in the fish tested in the current study was noted. The lowest *F*<sub>C</sub> was observed in April (the spawning period of the spring spawning *C. h. membras*), whereas the highest was noted in October (at the end of the intensive feeding period of spring spawning *C. h. membras*). Similar seasonal variation in *F*<sub>C</sub> was reported in *C. h. membras* from the eastern part of the Baltic Sea (Aro *et al.*, 2000), in Norwegian *C. harengus* (Hamre *et al.*, 2003) and in
**FATTY-ACID PROFILES IN C. HARENGUS MEMBRAS**

C. harengus from the North Sea. In these studies, the highest $F_C$ was also measured during the autumn. Similar studies were conducted for *S. pilchardus* and *E. encrasicholus* from the Mediterranean Sea by Zlatanos & Laskaridis (2007), who observed that the highest $F_C$ in *S. pilchardus* is between spring and early summer, whereas in *E. encrasicholus* it was noted between the end of winter and spring. These results support previous statements (Love, 1988) that fat is not metabolized continuously but gradually, and this is an indication that fish build energy reserves before spawning, during which these reserves are spent rapidly.

**FATTY-ACID PROFILE**

Similar to *C. h. membras* from the eastern Baltic Sea (Aro *et al*., 2000) *S. smaris* and *E. encrasicholus* caught in the Mediterranean Sea (Zlatanos & Laskaridis, 2007), in the *C. h. membras* tested in the current study, the dominant SFA was palmitic acid C16:0, that among MUFA it was oleic acid C18:1n−9c and that among PUFA it was docosahexaenoic acid C22:6n−3. In percentage terms, PUFA predominated in the lipid extracted from the muscle tissue of the fish tested in the current study.

Different fatty-acid profiles were observed in *C. harengus* from the North Sea (Aidos *et al*., 2002). Independent of the season or gonad maturation stage, Aidos *et al*. (2002) reported that, in percentage terms, MUFA were predominant in the fat of *C. harengus*. The total amount of MUFA varied between 34·9 and 58·8%, and, in addition, C22:1 was the predominant fatty acid, the content of which varied between 14·0 and 28·0%. High levels of that fatty acid are typical of *C. harengus* from the North Sea, and, according to data from the literature (Aidos *et al*., 2001), it originates from the main fatty acids in copepods (Ackman, 1982).
The main acids of the PUFA fraction in the fat extracted from the muscle tissue of the *C. h. membras* in the current study were EPA and DHA. Their percentage content and concentrations expressed in mg g\(^{-1}\) tissue changed throughout the year. DHA content was significantly higher than EPA throughout the study period. Bandarra *et al.* (1997) reported that there is a reduction in DHA content in *S. pilchardus* in April with a proportionate increase in EPA, which is attributed to a diversified diet. Since DHA is an important component of the structural membranes, the relative percentage of this acid can decrease in April during the postspawning period since it is used, for example, by females for egg formation. EPA, however, is the main fatty acid of plankton (Bandarra *et al.*, 1997), and the level of this acid remains more stable since deficits are replenished continually through feeding. Generally, during the postspawning period (when the fish are spent or when they begin to rebuild their energy resources), the content of combined EPA and DHA was the lowest. It is widely known that fats are mobilized when fishes are starved or when they mature (Love, 1988) since sexual maturation necessitates exploiting relatively large amounts of the energy obtained from food in order to develop sexual products. At the same time, selected fatty acids (higher proportions of PUFA than MUFA) are mobilized during spawning development (Henderson & Almatar, 1989). Love (1988) concluded that DHA did not tend to mobilize from muscle cells until the fat content in the muscles was extremely low, which would be in the late starvation period.

The decrease in EPA and DHA content in the postspawning period and corresponding increases during the intensive feeding period observed in the current study concur with the observations of Bandarra *et al.* (1997). The decrease of EPA and DHA content between November and April might be associated with the fish adapting to utilize these fatty acids for ovarian development (Schwalme *et al.*, 1993). The current study revealed that the percentage content of DHA in total lipid fraction of the samples analysed was correlated with the two variables \(M_S\) and \(F_C\), while the stronger correlation was observed for the variable \(M_S\). The regression equation explained 50% of the total variation of the dependent variable. This also indicates that other factors determine the variability of the percentage content of these acids in *C. h. membras*. One such factor is food preferences. It was proved that for *C. h. membras* from the southern Baltic, food preferences are related to specimen size and season, because, as reported by Szypuła *et al.* (1997) and Casini *et al.* (2004), food preferences vary depending on different season and different *C. h. membras* length categories. This explains the changes of fatty-acid profiles in relation to \(L_T\) classes that were noted in the current study.

Beltran *et al.* (1991) postulated that the content of PUFA (\%) in fishes decreases as \(F_C\) increases. This observation, however, has not been reported by other authors for *C. h. membras* (Linko *et al.*, 1985). Nevertheless, Zlatanos & Laskaridis (2007) reported a negative correlation between \(F_C\) and seasonal variations of \(n-3\) fatty acids content in *S. pilchardus* and *E. encrasicholus* from the Mediterranean Sea. *Sardina pilchardus* and *E. encrasicholus* are members of the Clupeidae family. A negative correlation between \(F_C\) and \(n-3\) fatty acids (\%) could be a feature of these species since Shirai *et al.* (2002) provided a similar correlation for *S. melanostictus*. On the other hand, the positive correlation between \(F_C\) and SFA observed by Zlatanos & Laskaridis (2007) implies that there are differences in the biological functions of various fatty acids in fishes. SFA probably serve as stored energy, which is why their concentrations increase during the intense feeding period.
The ratio of n−3:n−6 fatty acids in *C. h. membras* caught in the southern Baltic was high (5·67 ± 1·59) and depended on *LT*; it was higher in smaller specimens than in larger ones. This variation is linked to the feeding preferences of individuals of different ages. Younger individuals feed on nanoplankton, which is characterized by high levels of EPA (n−3 family of EUFA) (Lavaniegos *et al.*, 1997). In addition, larger individuals are characterized by the lowest contents of EPA and DHA (from the n−3 family) during the postspawning season since these acids are utilized for, among other purposes, egg development (Schwalme *et al.*, 1993).

Compared to the present study, a higher proportion of n−3:n−6 ratio of fatty acids was reported by Zlatanos & Laskaridis (2007) in *S. plichardus* (mean 13·0) and in *E. encrasicholus* (mean 13·7). Conversely, much lower n−3:n−6 ratio was measured in *S. smaris* (mean 1·57).

The current research indicated seasonal variations in *FC* and fatty-acid profile of *C. h. membras* samples; however, these were not as significant as those described by other authors for *C. h. membras* (Aro *et al.*, 2000), *C. harengus* from the North Sea stock (Aidos *et al.*, 2002) or *C. harengus* from the Norwegian stock (Hamre *et al.*, 2003). In the current study, the highest seasonal variation was observed for PUFA content, which emphasizes the significant role they play in the maturation process. The changes observed in fatty-acid profiles were determined by factors such as *MS* and *FC*, which, in turn, depended on fishing season. The sex of the specimens studied, however, was not noted to influence changes in fatty-acid profiles.

The studies on seasonal variation of fish lipid content and fatty-acid profiles could provide information about fish physiology. It is important to note that the current study was conducted over the course of 2 years, whereas it is known that conditions in the sea, such as food availability and composition, change annually. Therefore, continuing the study for several years would be advisable. Moreover, simultaneous studies of the fatty-acid profiles of fish and plankton could make the results easier to interpret.

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