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Season and cooking may alter fatty acids profile of polar lipids from Blue-Back Fish

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Abstract

Polar lipids (PoL) represent a new promising dietary approach in the prevention and treatment of many human diseases, due to their potential nutritional value and unique biophysical properties. This study investigates the effects of catching season and oven baking on the fatty acid profiles (FAP) of PoL in four species of blue-back fish widely present in the North Adriatic Sea: anchovy (*Engraulis encrasicholus*), sardine (*Sardina pilchardus*), sprat (*Sprattus sprattus*) and horse mackerel (*Trachurus trachurus*).

PoL levels (427–652 mg/100 g flesh) varied among the four species, with no significant seasonal variations within species. FAP of raw fillets were particularly high in PUFA, especially DHA and EPA; total PUFA was constant in all species throughout the year, while n-3 PUFA rose in spring (except in sprat), especially due to the contribution of DHA. The FAP response for PoL to oven baking was species-specific and, among n-3 PUFA, DHA exhibited the greatest heat resistance; the influence of oven baking on FAP was found to be correlated with the catching season, especially for anchovy and sardine, while sprat PoL were not affected by cooking processes.

The four species analyzed in this study presented very low n-6/n-3 fatty acid ratios and highly favorable nutritional indices, emphasizing their PoL qualities and promoting their role in increasing human n-3 PUFA intake. The four species can be considered as superior sources of n-3 PUFA and can be employed as supplements in functional food manufacturing and in pharmaceutical and cosmetic industries.

Keywords

Blue-back fish · Polar lipids · Neutral lipids · Fatty acid composition · Season influence · Cooking effects · Baking · Nutritional quality

40 **Abbreviations**

41 AI	Atherogenicity index
42 ALA	α-Linolenic acid
43 ARA	Arachidonic Acid
44 DHA	Docosahexaenoic acid
45 EPA	Eicosapentaenoic acid
46 FAME	Fatty acid methyl esters
47 FAP	Fatty acid profile
48 HH	Hypocholesterolemic to hypercholesterolemic fatty acid ratio
49 LNA	Linoleic acid
50 MUFA	Monounsaturated fatty acid(s)
51 n-3 PUFA	Long-chain n-3 polyunsaturated fatty acid(s)
52 n-6 PUFA	Long-chain n-6 polyunsaturated fatty acid(s)
53 NL	Neutral lipids
54 PL	Phospholipids
55 PoL	Polar lipids
56 PUFA	Polyunsaturated fatty acid(s)
57 SFA	Saturated fatty acid(s)
58 TI	Thrombogenicity index
59 TL	Total lipids

60

Introduction

Fish is not only a valuable source of high quality animal protein, but it is also well known that fish lipids are rich in long-chain n-3 polyunsaturated fatty acids (n-3 PUFA), especially 20:5n-3 (EPA) and 22:6n-3 (DHA); these fatty acids are unanimously considered as molecules with high nutritional value for the human diet and represent a focus of nutritionists, for the prevention and treatment of obesity (Fernandes et al., 2014; Parmentier, Al Sayed Mahmoud, Linder, & Fanni, 2007; Schneedorferová, Tomčala, & Valterová, 2015). Several studies have demonstrated that n-3 PUFA may be considered as nutraceuticals, i.e. medicinal foods that play a role in maintaining well-being, enhancing health and modulating immunity and thereby able to prevent as well as to treat specific diseases such as cardiovascular diseases, rheumatoid arthritis, diabetes, ulcerative colitis, allergies, eczema, thickening of the skin, weight gain, premature labor, depression and cancer (Fernandes et al., 2014; Hossain, Hosokawa, & Takahashi, 2009; Loftsson, Ilievska, Asgrimsdottir, Ormarsson, & Stefansson, 2016; Murakawa et al., 2007; Taylor, Pletschen, Arends, Unger, & Massing, 2010).

The fatty acid profile (FAP) of marine fish can vary among species and among individuals, depending on their diet, size, age, gender, environmental conditions, season and method of capture (Fernandes et al., 2014).

Several studies have observed that the total lipid (TL) content of fish and the FAP may vary throughout the year, due to exogenous and endogenous factors, and may also be affected by processing or cooking methods (Kaçar & Başhan, 2015); in fact, cooking may significantly alter the content, composition, biological activity and nutritional value of fish lipids, due to water loss, oxidation, loss of fatty acids by leaching, and fatty acid exchange between fish and other oils (Little, Armstrong, & Bergan, 2000). The FAP of TL is influenced by the lipid classes by which they are constituted: neutral lipids (NL) and polar lipids (PoL), which are different in composition and role. NL mainly consist of triacylglycerols, storage lipids used for energy purposes, for the maturation of gametes during the breeding season and as a temporary PUFA reservoir which can be forwarded to structural lipids or directed to specific metabolic pathways (Varljen, Baticic, Sincic-Modric, Obersnel, & Kapovic, 2004). The PoL include mainly phospholipids (PL) (De Leonardis & Macciola, 2004; Fanni, Linder, & Parmentier, 2004), important structural components of cell membranes and eicosanoid precursors. The

89 FAP of NL seems to depend mainly on the quality and availability of dietary intake and on the
90 reproductive status of the subject, while the PoL fraction is less representative of dietary composition
91 and can vary mainly due to environmental conditions such as temperature and salinity (Cordier, Brichon,
92 Weber, & Zwingelstein, 2002).

93 The n-3 PUFA of PoL are important precursors of beneficial prostaglandins and platelet-activating
94 factors and have a number of interesting biological activities, ranging from involvement in inflammatory
95 and allergic reactions and antihypertensive responses to physiological processes (reproduction, fetal
96 development, childbirth) (Blank, Cress, Smith, & Snyder, 1992). Conversely, oxylipins formed from n-
97 3 and n-6 PUFA have essential roles in normal physiology and function, but can also have detrimental
98 effects (Gabbs, Leng, Devassy, Monirujjaman, & Aukema, 2015). In mammalian systems, longer-chain
99 n-3 and n-6 PUFA are synthesized from two essential precursors: α-linolenic acid (ALA; 18:3 n-3),
100 leading to n-3 PUFA, and linoleic acid (LNA; 18:2 n-6), resulting in n-6 PUFA. The precursors compete
101 for the enzymatic system, thus a high intake of ALA supports the elongation of n-3 PUFA and reduces
102 the elongation of n-6 PUFA (Brenner, 1977). The n-6/n-3 PUFA ratio is the main important index of
103 nutritional value of fish lipids; the intake of an appropriate ratio of essential fatty acids appears to be
104 critical for the prevention and reduction of obesity, and nutritionists recommend human intakes of PUFA
105 with ratios below 4 (Santos-Silva, Bessa, & Santos-Silva, 2002). Currently, the n-6 PUFA content in the
106 human diet is from 3.75 to 10 times higher than recommended value, which is strongly correlated to an
107 increased incidence of chronic non-transmissible diseases. Western diet, composed of high contents of
108 red meat, refined flours and industrial products (with high levels of n-6 precursors and low levels of n-
109 3 ones), enhances the production of long-chain n-6 PUFA and suppresses the production of n-3 PUFA
110 (Adkins & Kelley, 2010; Fernandes et al., 2014). Moreover, the amount of DHA available for the global
111 body metabolism remains insufficient for a large proportion of consumers (Parmentier et al., 2007), so
112 that a higher dietary intake of n-3 and a reduction of n-6 consumption is strongly recommended for the
113 correction of an unbalanced diet (Fernandes et al., 2014).

114 Currently, there is increasing attention on marine PoL, especially on PL, because of their potential
115 nutritional value and their unique biophysical properties, so that their administration seems to be a
116 promising new dietary approach in the prevention and treatment of many human diseases. Compared to

117 NL, PL of marine origin show greater nutritional value, higher bioavailability and higher intestinal
118 absorption of n-3 PUFA, properties due to the esterification in the sn-2 position of ARA, EPA and DHA
119 (Parmentier et al., 2007); PL also present better resistance to oxidation compared to triacylglycerols
120 from the same source (Adkins & Kelley, 2010). Fish species with a high PL content are considered
121 valuable food products due to their high levels of PUFA. Previous studies suggested that fish meals rich
122 in PL could be used as a valuable functional ingredient of food or nutraceuticals (Henna Lu, Nielsen,
123 Timm-Heinrich, & Jacobsen, 2011). The demand for PUFA in health-related products is increasing;
124 modern biotechnology and engineering make it possible to design and purify marine PL for a wide range
125 of applications (e.g. in nutrition as dietary supplements, and in drug delivery in the form of liposomes)
126 (Loftsson et al., 2016; Mika, Swiezewska, & Stepnowski, 2016). Despite the nutritional, pharmaceutical
127 and cosmetic value of marine PL having been widely discussed (Burri, Hoem, Banni, & Berge, 2012),
128 there are very limited studies on the FAP of PoL in blue-back fish, including species widely caught and
129 commonly consumed; these small pelagic fish may provide a valuable source of n-3 PUFA due to the
130 abundance of the stocks. The aim of this study was the market promotion of these species, currently
131 with a low commercial value, and the divulgation of their nutritional properties. Moreover, since these
132 species analyzed are generally consumed cooked in the area subjected to study, the effects of cooking
133 process have been assessed. An increment in the consumption of local fish in place of imported or over-
134 exploited fish species would represent an example of sustainable utilization of the natural marine
135 resources, and would also have a positive environmental impact (Guo, Vikbjerg, & Xu, 2005). Therefore,
136 the objectives of this study are: (1) characterizing the PoL content in four species of blue-back fish from
137 the Adriatic Sea; (2) highlighting any changes in PoL content and FAP due to the seasonal period and/or
138 cooking process; (3) providing information about the best season for catching and consuming the fish
139 species considered.
140

141 ## Materials and Methods

142 ### Sample Collection and Preparation

143 Four fish species were included in this study: anchovy (*Engraulis encrasicholus*), sardine (*Sardina*
144 *pilchardus*), sprat (*Sprattus sprattus*) and horse mackerel (*Trachurus trachurus*). Fish caught in the

145 North Adriatic Sea were obtained from wholesale fish markets in Cesenatico and Rimini, in three
146 different seasons, fall (four batches for anchovy, sardine and horse mackerel) or winter (three batches
147 for sprat), and in the following spring, as described in the study of Pirini, Testi, Ventrella, Pagliarani
148 and Badiani (2010). Within each batch, one fillet out of each of 30 specimens was retained to constitute
149 the pooled raw reference.

150 The contralateral fillets were oven-baked as described by Pirini, Testi, Ventrella, Pagliarani and
151 Badiani (2010). Briefly, the oven temperature was set at 190°C; cooking time ranged from a minimum
152 of 5 min for sprat to a maximum of 9 min for horse mackerel to reach a core temperature of 70 °C, as
153 checked by an iron–constantan thermocouple connected to a digital potentiometer (Type J/K
154 Thermometer mod. 421502, Extech Instruments Corp., Waltham, MA, USA).

155 Raw and cooked fillets were either skinned (sardine, sprat and horse mackerel) or left unskinned
156 (anchovy), as customarily prepared in Italy, then cut into small pieces, homogenized using a stainless-
157 steel meat mincer, and stored at −20 °C until lipid analysis.

158

159 **Lipid Analysis**

160 Lipid analyses were carried out in duplicate on 5 g of homogenized fillet, both raw and cooked. TL
161 were extracted according to the method of Folch, Lees and Sloane Stanley (1957) and weighed after
162 complete evaporation of the solvent. TL were separated into NL and PoL fractions by SPE (solid phase
163 extraction) using Phenomenex Strata SI-1 Silica normal phase columns (55 µm, 70 A) (Torrance, CA,
164 USA) with a capacity of 12 mL, containing 2 g silica, according to the method described by Bayır et al.
165 (2010), with some modifications as shown below. The sample of TL diluted in 3 mL of chloroform was
166 applied to the column equilibrated by four aliquots of 3 mL of chloroform. The NL fraction was eluted
167 by adding 3 mL of chloroform eight times with a flow of two drops per second; PoL were eluted by four
168 aliquots of 3 mL of methanol and four aliquots of 3 mL of a chloroform/methanol mixture (3:7 v:v),
169 with a flow of two drops per second. PoL content was evaluated gravimetrically after completely solvent
170 evaporation. Colorimetric determination of phosphorus content confirmed gravimetrical results, thus
171 confirming that the PoL fraction coincided with PL (Bayır et al, 2010). Fractions obtained were weighed,
172 diluted in a specific volume of chloroform and methanol in the ratio 1:1, and stored at −20 °C prior to

173 use for further analysis. The methyl esters of the PoL fatty acids were prepared by transmethylation with
174 acid catalyst (Christie, 1989) and analyzed on a Varian 3380 gas chromatograph equipped with a J&W
175 Scientific DB-23 fused silica capillary column (30 m × 0.25 mm), under the conditions reported by
176 Pirini et al. (2010).

177 Fatty acid identification was accomplished by comparing the retention time of unknown FAME with
178 those of known FAME standard mixtures (Sigma-Aldrich Corp., St. Louis, MO, USA; PUFA No.1,
179 Marine Source, and PUFA No.3, Menhaden Oil, SUPELCO, Inc., Bellefonte, PA, USA).

180

181 **Lipid Quality Indices**

182 A qualitative assessment of the nutritional profile of the PoL fraction was done through computation
183 of the ratio between the percentages of n-6 and n-3 PUFA, often highlighted as a key element for a
184 healthy diet (Simopoulos, 2006). Moreover, the nutritional quality of the PoL fraction was assessed by
185 three indices: atherogenicity index (AI), thrombogenicity index (TI) and hypocholesterolemic to
186 hypercholesterolemic fatty acid ratio (HH), as described by Ulbricht and Southgate (1991) and Santos-
187 Silva, Bessa, & Santos-Silva (2002). AI indicates the relationship between the sum of the main saturated
188 fatty acids (14:0 and 16:0) and that of the main classes of unsaturated fatty acids (MUFA and n-3 and
189 n-6 PUFA); AI was calculated by applying the following equation:

190 $$\mathrm{AI} = \frac{[(4 \times 14{:}0) + 16{:}0]}{\mathrm{MUFA} + \mathrm{n} - 6 + \mathrm{n} - 3}$$

191 IT is expressed as the relationship between pro-thrombogenic (saturated) and anti-thrombogenic fatty
192 acids (MUFA, n-6 and PUFA) and was calculated by applying the following equation:

193 $$\mathrm{TI} = \frac{(14{:}0 + 16{:}0 + 18{:}0)}{(0.5 \times \mathrm{MUFA}) + (0.5 \times \mathrm{n} - 6) + (3\,\mathrm{n} - 3) + \frac{\mathrm{n} - 3}{\mathrm{n} - 6}}$$

194 The HH ratio is related to cholesterol metabolism and was calculated according to the following
195 equation:

196 $$\mathrm{HH} = \frac{[(18{:}1\mathrm{n} - 9 + 18{:}2\mathrm{n} - 6 + 18{:}3\mathrm{n} - 3 + 20{:}5\mathrm{n} - 3 + 22{:}5\mathrm{n} - 3 + 22{:}6\mathrm{n} - 3)]}{(14{:}0 + 16{:}0)}$$

197

198 **Analytical Quality Assurance**

The analytical quality was controlled for three times by analyzing the standard reference material "Meat Homogenate" (SRM 1546; NIST, Gaithersburg, MD) for lipid content as well as FAP, following the same analytical procedure used in this work. The means determined were always within the certified (or reference) intervals.

Statistical Analysis

For each species, data (lipid content and FAP, expressed as mole%) were arcsin-transformed and analyzed using a two way "between group/within subjects" analysis of variance (ANOVA). "Season of catch" (fall/winter vs spring) was the between-group factor, whereas "State" (raw vs cooked) was the within-subjects (repeated measures) factor. Means were separated at, or below, the 5% probability level using Tukey's HSD *post hoc* test. All statistical computations were performed using the Statistica® software package (Release 7, 2005; StatSoft Inc., Tulsa, OK, USA).

Results

Polar Lipids Content

The percentages of PoL in raw and cooked fillets of the blue-back fish examined are shown in Table 1. Levels of PoL, calculated as g/100 g of TL (or %PoL) in fresh fillets, ranged from 4.83% (sprat in spring) to 22.8% (sardine in spring). In sardine, these values varied significantly among fishing seasons: they were higher in spring, when the lower amount of TL characterizes this species in this period. The same parameters expressed in absolute terms (mg of PoL/100 g of raw fillet) showed levels between 427 and 652 mg/100 g tissue, with no statistically significant seasonal variation within species, suggesting variability in TL profile during the year.

The cooking process significantly influenced total PoL content (expressed in absolute terms) in all species, due to the loss of water. The %PoL of anchovy increased significantly in both seasons, while in sardine and in horse mackerel this effect was only appreciable in subjects collected in fall.

Fatty Acid Profile of Polar Lipids in Raw Samples

226 The FAP of PoL fractions of the four species analyzed in fall/winter and in spring is shown in Tables
227 2 and 3. The n-3 PUFA were the major fraction, with percentages ranging between 42.40% and 57.06%,
228 followed in decreasing order by SFA (26.70-30.85%), MUFA (8.58-13.89%) and n-6 PUFA (2.85-
229 8.28%). The SFA were high in palmitic acid (16:0), with a peak of 35,40% in the spring in sardine;
230 among the MUFA, oleic acid (18:1 n-9) was predominant, with values ranging between 2.90% and
231 6.75%; ARA (1.07-3.70%) was the most represented n-6 PUFA, while DHA (30.50-45.23%) and EPA
232 (4.67-13.22%) were the major ones among n-3 PUFA. The species that contains the highest PUFA
233 content is horse mackerel although sprat is richer in n-3 PUFA, followed by horse mackerel and anchovy
234 fished in spring.
235
236 **Seasonal Dynamics of Fatty Acid Profile of Polar Lipids**
237 The SFA, MUFA and PUFA composition of PoL (Tables 2 and 3) generally showed a remarkable
238 pattern of seasonality, reflecting fatty acid fluctuations. Almost all SFA tended to decline from fall to
239 spring, i.e. the 14:0 in sardine (from 3.98% to 0.68%, about 1/6 of the fall value). An exception is the
240 increase of 16:0 content in sardine (from 18.0% in fall to 31.2% in spring) and, to a lesser extent, in
241 anchovy (from 20.39% in fall to 35.40% in spring). However, these changes had no effect on the overall
242 value of SFA, except in sardine (from 30.85% in fall to 41.73% in spring). Significant variations in the
243 percentages of MUFA were found in anchovy, sardine and horse mackerel caught in fall, mainly due to
244 the increase in both 16:1n-7 and 18:1n-9. In these very same species, seasonal changes in fatty acid
245 composition of MUFA were also recorded for NL. Furthermore, fall-caught horse mackerel showed an
246 increase in SFA (especially 16:0), as it can be seen in Table 5 and Table 6.
247 The percentage of total PUFA, instead, did not change seasonally: in anchovy, sardine and horse
248 mackerel caught in fall, the reduction in DHA content was compensated for by increases in the
249 percentage of EPA or of n-6 PUFA. In fact, anchovy and sardine were richer in n-6 PUFA in fall, while
250 in sprat and in horse mackerel seasonal rates remain unchanged. The levels of n-3 PUFA were
251 significantly higher in spring, except for sprat. In anchovy, this trend reflected that of the major n-3
252 PUFA (EPA and DHA), while in sardine and horse mackerel it was connected to a marked increase in
253 DHA, which compensated for the significant decline of EPA in spring. A similar pattern was observed

254 for NL PUFA, where higher levels of n-6 are detected in anchovy caught in fall and increased n-3 in
255 spring horse mackerel (see Table 5 and Table 6).
256
257 **Effect of Cooking on Fatty Acid Profile of Polar Lipids**
258 Upon cooking, significant changes in percentages of fatty acids, connected to season and species,
259 were frequently observed (see Tables 2 and 3). As was observed for raw samples, the n-3 PUFA fraction
260 was the highest, with percentages ranging from 39.38% to 57.38%, followed by SFA (26.73-38.10%),
261 MUFA (5.93-11.75%) and n-6 PUFA (2.69-7.99%). In particular, cooking seemed to cause a significant
262 reduction in SFA level in spring sardine, mainly due to the remarkable decrease of 16:0; on the contrary,
263 in fall in anchovy, and in spring in horse mackerel, the same fatty acid tended to achieve notably higher
264 values. In sprat and horse mackerel, the quantities of SFA were less influenced by cooking and season,
265 while in anchovy and in sardine the interaction (Sc*St) between season and status was significant. The
266 cooking process induced an important decrease in MUFA content in sardines, particularly those
267 collected in fall; on the contrary, an increase could be observed in anchovy. Cooking caused a significant
268 drop in total PUFA only in the fall anchovy. For n-6 PUFA, the cooking effect seemed to consist of a
269 small decrease in the total concentration in horse mackerel fished in spring and in anchovy and sardine
270 fished in fall, in which the interactions Sc*St are significant for many fatty acids. The effects of the
271 cooking process on n-3 PUFA mainly involved fall anchovy and sardine; in the former species, cooking
272 caused significant loss of DHA and thus a significant decrease in n-3 PUFA; in the latter, all n-3 PUFA,
273 excluding DHA, incurred significant losses, while in spring, EPA and DHA appeared to increase.
274 NL showed a different behavior towards cooking processes compared to PoL; in each species
275 analyzed, SFA, MUFA and n-6 PUFA from NL are quite stable, or they can be even found concentrated
276 in cooked fillet, due to the water loss. On the contrary, the trend of n-3 PUFA is similar to that of PoL,
277 since drops are recorded in fall anchovy and spring sardines, mainly due to loss in DHA (see Table 5
278 and Table 6).
279
280 **Nutritional Indices**

281 The n-6/n-3 PUFA ratios (see Tables 2 and 3) were steadily below the value of 1 (0.05 to 0.16), given
282 the clear predominance of n-3 PUFA. In particular, a seasonal reduction in n-6/n-3 ratio was found in
283 raw anchovy (0.11 in fall to 0.06 in spring), while a less important decrease was shown for horse
284 mackerel (0.16 to 0.12). Cooking finally produced slight but significant increases in n-6/n-3 ratio only
285 in fall anchovy.

286 Table 4 shows the nutritional profile of PoL of the four species in the two seasonal periods in the raw
287 and cooked states. Within each species, the comparison between seasons did not reveal significant
288 differences in AI and in TI. The values of AI in subjects in the raw state varied from a minimum of 0.21
289 in horse mackerel to a maximum of 0.56 in sardine, both species caught in spring; in sprat (in both
290 seasons) and in anchovy caught in spring, the TI were in the range 0.11–0.25, respectively. After baking,
291 the values of AI were included in the range 0.23 to 0.59, respectively in spring sprat and in fall sardine.
292 Within each species, comparison between seasons showed a significant decrease of AI in spring anchovy
293 and sardine only after cooking. Finally, the highest HH value observed was 4.49 in spring horse
294 mackerel; the lowest was 1.64 in spring sardine. In raw anchovy and sardine, HH values tended to be
295 slightly higher in fall. In all other cases, the differences between seasons and between raw and cooked
296 fish seemed to be minor.

297

298 **Discussion**

299 **Seasonal Effect on Polar Lipids Content and Fatty Acid Profile**

300 The proportions of PoL (g/100 g of TL) did not differ from those reported in the literature (De
301 Leonardis & Macciola, 2004; Fanni, Linder, & Parmentier, 2004; Shahidi & Miraliakbari, 2006): the
302 highest values of PoL were detected during the "lean period", when the amount of NL in the flesh
303 decreases in favor of gonadal maturation. Even the amount of PoL agrees by and large with the data
304 from other authors (Kolakowska, Olley & Dunstan, 2006), ranging between 300 and 500 mg/100 g of
305 flesh (see Table 1). The lack of significant seasonal variation in PoL content confirms that the variations
306 in TL detected in the course of the year should be mostly attributable to cyclical variations of NL, as
307 reported by other authors (Fanni, Linder & Parmentier, 2004). As far as we know, this is one of the very

308 few studies reporting seasonal changes of PoL fatty acids of blue-back fish from the Adriatic Sea;
309 therefore the comparison with literature data is fragmented and incomplete.

310 Overall, the results of this survey indicate that the FAP of raw PoL exhibits a preponderance of PUFA,
311 especially DHA and EPA. Total PUFA remains substantially constant in all species during the year,
312 while the n-3 PUFA content (except in sprat) tends to rise in spring, especially due to the contribution
313 of DHA. Furthermore, n-6 PUFA show significant seasonal variations only in anchovy and sardine, with
314 lower values in spring compared to fall. These findings are consistent with the results obtained in the
315 study by Passi et al. (2002), where the FAP of PoL were analyzed in sardine and horse mackerel from
316 the central Tyrrhenian Sea, without specifying the season of capture. Other investigations on PoL from
317 *Anguilla anguilla* reported low levels of MUFA and very high levels of PUFA, mostly n-3 (Ciappellano,
318 Erba, Colombo, Testolin & Bolis, 1999). Similarly, in a study of seasonal fluctuations in FAP in three
319 anadromous subspecies (*Salmo trutta caspius*, *S. t. labrax* and *S. t. macrostigma*), Bayır et al. (2010)
320 reported PoL fractions with very high percentages of PUFA and SFA (due to the high levels of 16:0).
321 Seasonal changes in the various groups of fatty acids varied depending on the species considered, in
322 agreement with the lack of homogeneity occurring in changes of PoL in blue-back fish. Given the large
323 number of variables involved, it is difficult to distinguish the origin of PoL fluctuations, especially since
324 in aquatic animals this fraction was found to be susceptible to the dietary quality, although to a lesser
325 extent than NL (De Souza et al., 2008; Standal, Axelson & Aursand, 2010).

326 On this basis, the seasonal changes we observed in PoL of blue-back fish can be attributed to the
327 different seasonal availability of fatty acids in the aquatic food chain rather than homeoviscous
328 adaptation. On the other hand, high levels of n-3 PUFA, typical in winter or early spring due to the
329 adaptation of aquatic animals at low temperatures, were detected only in horse mackerel.

330

331 **Effect of Cooking on Fatty Acid Profile**

332 Important changes in the FAP of fish muscle, caused by culinary treatments, consist of considerable
333 loss of DHA and EPA and depend on the cooking method (Larsen, Quek & Eyres, 2010; Türkkan, Cakli
334 & Kilinc, 2008). Baking is considered by many authors the best heat treatment for preservation of all
335 lipid features of fish meat, including the PUFA content and n-3/n-6 ratio (García-Arias et al., 2003); it

336 has been found to be the best preparation technique with regard to retention of EPA and DHA of TL,
337 maintaining over 80% for both fatty acids (Al-Shagir et al., 2004; Mierke-Klemeyer et al., 2008). The
338 great increase of PoL content in cooked fillets (see Tables 2 and 3) when compared to the raw
339 counterpart is rationally connected to the loss of moisture and storage lipids, subjected to melting or
340 leaching from the flesh with the so-called "cook-out" (Little, Armstrong, & Bergan, 2000).

341 The heat resistance of many fatty acids found in this study, including n-3 PUFA (especially in spring
342 sardine, sprat and horse mackerel), can be related to some extent of "internal protection" of n-3 fatty
343 acids that prevent the degradation of PUFA during heat treatments. This protective effect may be due to
344 the presence of NL and PoL incorporated in lipoprotein structures, such as biological membranes
345 (Küllenberg, Taylor, Schneider, Massing and Ulrich, 2012), and to the presence of high levels of natural
346 antioxidants (vitamin E, astaxanthin) in the flesh (Gladyshev et al., 2006; Larsen, Quek & Eyres, 2010).
347 From the results of this study (Tables 2 and 3), the response to baking is species-specific, as also reported
348 by other authors (García-Arias et al., 2003; Schneedorferová, Tomčala & Valterová, 2015); it seems to
349 be correlated to the catch season in anchovy and sardine: in baked anchovy caught in fall, significant
350 increments in the percentages of SFA, MUFA and n-6 PUFA were detected, together with a decrease in
351 the global percentage of PUFA (due to significant reductions in n-3 PUFA), and a consequent increase
352 in n-6/n-3 ratio. In baked sardine, significant increments were detectable in the MUFA and n-6 PUFA
353 percentages of subjects caught in fall. These findings agree with those reported in the study of Martelli
354 et al. (2013), in which the FAP was influenced by both catch season and cooking treatments.

355

356 **Nutritional Indices**

357 The n-6/n-3 ratio has been suggested to be the best index for comparing the relative nutritional value
358 of fish oils (Simopoulos, 1991). The typical Western diet is characterized by a high intake of n-6 PUFA
359 and a low intake of n-3 PUFA. According to the recommendations of nutritional advisers, the attempt
360 is to increase the levels of n-3 PUFA in the diet, such that the n-6/n-3 ratio does not exceed 4.0 (Santos-
361 Silva, Bessa, & Santos-Silva, 2002). Analysis of PoL in the fish species included in this study reported
362 an n-6/n-3 ratio between 0.05 and 0.17 (see Tables 2 and 3), which are values 2-15 folder lower than
363 those reported by Simonetti et al. (2008) in a study of PoL in four freshwater species; these findings

364 suggest excellent nutritional quality in terms of FAP of PoL in the fish sources investigated, also
365 confirmed by the AI and indices always lower than 1 (between 0.10 and 0.59, among raw and cooked
366 samples). These indices (see Table 4) indicate the potential for stimulating platelet aggregation and are
367 primarily related to a decrease in cardiovascular disease risk; thus, the smaller the values, the greater the
368 protective potential for coronary artery disease (Turan, Sönmez & Kaya, 2007). From the findings of
369 this study, sardine was the species with the highest index values. AI and TI indices were not influenced
370 by seasonality or by cooking, with the exception of cooked sardine and anchovy which showed a
371 decrease of both AI and TI indices in spring compared to the raw state.

372 The HH index refers to the ratio between the sum of hypercholesterolemic fatty acids and the sum of
373 hypercholesterolemic fatty acids, and indicates the specific effects of fatty acids on the cholesterol
374 metabolism; from a nutritional point of view, high HH values are considered beneficial to human health
375 (Testi, Bonaldo, Gatta & Badiani, 2006). HH index values from this study (means ranging from 1.64 to
376 4.49, see Table 4) were higher than those obtained in similar investigations on black needle, white needle,
377 mackerel and sardine from a Brazilian market (Fernandes et al., 2014). HH indices tended to be higher
378 in horse mackerel and sprat and showed significant seasonal variability in raw and cooked sardine;
379 furthermore, in every species analyzed, they exhibited a decrease after the cooking process, with the
380 exception of sprat, for which baking increased the index.

381 Due to the very low n-6/n-3 fatty acid ratios and the highly favorable nutritional indices found in the
382 blue-back fish analyzed in this study, the PoL qualities of the four species examined may play an
383 important role in the human diet, helping to increase significantly the n-3 fatty acid intake and improving
384 consumer health (Hossain, Hosokawa & Takahashi, 2009; Murakawa et al., 2007; Ulbricht & Southgate,
385 1991; Taylor et al., 2010). Furthermore, the existence of such high levels of EPA and DHA could make
386 blue-back fish PoL biologically valuable in the food industry for enrichment and production of
387 functional foods with higher nutritional value (Burri, Hoem, Banni, & Berge, 2012). This study
388 contributes to finding new commercialization strategies for these species, possibly as value-added
389 products. This study promotes the importance of marine PoL for the human diet, as a valuable source of
390 PUFA, and highlights the nutritional quality of four popular blue-back species from the Adriatic Sea (*E.*
391 *encrasicholus*, *S. pilchardus*, *S. sprattus* and *T. trachurus*). This manuscript presents, for the first time,

392 data on the influences of catch season and cooking treatments on the FAP of PoL, and therefore on the
393 nutritional quality of the species analyzed. Sprat was the only species not affected by seasonal variations
394 or by cooking processes. For the other species, especially sardine and anchovy, it is difficult to delineate
395 the best season for capture and consumption because, although the data indicate a higher level of PUFA
396 (in particular n-3) in the specimens caught in spring, the nutritional indices of cooked samples were
397 generally higher in those collected in fall.

398 The increased accessibility of marine lipids during recent years has opened up new possibilities for
399 the use of PoL not only as a superior nutritional source of n-3 PUFA, but also for use in the
400 pharmaceutical, cosmetic and functional food industries (Burri, Hoem, Banni, & Berge, 2012). Since
401 the only available source of n-3 PUFA comes at present from marine lipids, there is a need to scale-up
402 productions of these compounds and to identify new affordable resources, natural or synthetic (Løvaas
403 et al., 2006); future investigations should explore the PoL content in other marine species, krill, fish roe,
404 fish by-products (viscera and skin) and even in non-animal marine sources such as microalgae, in order
405 to meet the growing demands of the market. In addition, in order to complete the assessment of the role
406 of PoL in food manufacturing and in pharmaceutical and cosmetic applications, other important aspects
407 which need to be considered and deepened are the bioavailability of PoL from different sources, and the
408 identification of reliable physiological markers to confirm and determine their bioactivity.

409

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413

414 **Conflict of interest**

415 The authors declare that there is no conflict of interest regarding the publication of this article.

416

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561

562 **Table 1** Polar lipid (PoL) content in blue-back fish fillets from the four species, in the raw and cooked

563 state, and from different seasons

Fish species	*Season*	PoL (g/100g TL)		PoL (mg/100g flesh)	
		Raw	*Cooked*	*Raw*	*Cooked*
Anchovy	*Fall*	y11.1 ± 2.25	x18.9 ± 1.61	y486 ± 24	x1028 ± 174
	Spring	y7.71 ± 1.31	x15.1 ± 2.12	y442 ± 56	x977 ± 81
Sardine	*Fall*	y6.70 ± 1.38b	x11.9 ± 1.46b	y486 ± 58	x827 ± 46
	Spring	22.8 ± 2.21a	24.9 ± 3.65a	y652 ± 68	x927 ± 71
Sprat	*Winter*	14.1 ± 4.22	25.9 ± 14.4	y482 ± 130	x967 ± 154a
	Spring	4.83 ± 0.43	6.08 ± 0.73	539 ± 31	662 ± 30b
Horse mackerel	*Fall*	y20.5 ± 5.41	x29.6 ± 3.58	y427 ± 34	x679 ± 122
	Spring	20.4 ± 4.56	25.0 ± 6.93	y595 ± 37	x845 ± 86

564

565 *Values are means of four determinations ± Std Dev; values in the same row, with trait preceded by*

566 *different letters (x, y), differ significantly ($p < 0.05$) for state (raw or cooked); values within a column,*

567 *followed by different letters (a, b), differ significantly ($p < 0.05$) for catch season.*

568

569 **Table 2** Content of selected fatty acids (mole%) in polar lipids from skin-on fillets of anchovy and

570 skinned sardine in the raw (R) and cooked (C) state (St)

Fatty acids [A, B, C]	St	**Anchovy**		Statistical analysis				**Sardine**		Statistical analysis			
		Fall	*Spring*	RSD	Sc	St	Sc*St	*Fall*	*Spring*	RSD	Sc	St	Sc*St
14:0	R	3.00a	1.29b	0.940	***	*	n.s.	x3.98a	0.68b	0.267	***	**	*
	C	3.30a	1.41b					y2.47a	0.65b				
16:0	R	x19.54b	25.83a	0.762	***	*	***	y20.39b	x35.40a	2.078	**	n.s.	***
	C	x23.23	24.51					x28.68	y27.95				
18:0	R	y5.46a	4.36b	0.384	***	n.s.	**	5.83	x5.26	0.451	**	*	**
	C	x6.60a	3.79b					6.21a	y3.76b				
Σ SFA[D]	R	y29.62	32.65	1.253	n.s.	*	**	30.85b	x41.73a	2.773	n.s.	n.s.	**
	C	x35.10a	30.84b					38.10	y32.72				
16:1n-7	R	2.71a	1.21b	0.092	***	*	n.s.	x3.26a	0.64b	0.181	***	**	**
	C	2.98a	1.28b					y1.99a	0.62b				
18:1n-9	R	y3.71a	x2.90b	0.069	***	n.s.	***	x6.19a	3.19b	0.524	***	**	n.s.
	C	x4.16a	y2.51b					y4.16a	2.73b				
18:1n-7	R	x2.94	x3.23	0.082	n.s.	n.s.	***	x2.66	x2.85	0.234	n.s.	**	n.s.
	C	x3.26	y2.90					y2.04	y2.17				
Σ MUFA[E]	R	y9.84a	x7.70b	0.167	***	n.s.	***	x13.89a	6.90b	0.901	***	**	*
	C	x11.16a	y6.93b					y8.94	5.93b				
18:2n-6	R	1.15a	0.54b	0.049	***	n.s.	n.s.	x1.29a	0.96b	0.083	*	***	**
	C	1.10a	0.53b					y0.79	0.88				
20:4n-6	R	2.20a	1.29b	0.032	***	n.s.	**	1.59a	1.07b	0.200	***	n.s.	n.s.
	C	2.12a	1.35b					1.64a	1.13b				
22:5n-6	R	x1.52a	0.96b	0.033	***	***	**	1.11	1.07	0.112	n.s.	n.s	n.s.
	C	y1.28a	0.93b					1.12	1.31				
Σ n-6 PUFA[F]	R	x5.76a	3.33b	0.053	***	***	**	x5.01a	3.78b	0.216	***	**	**
	C	y5.34a	3.29b					y4.08	3.91				
18:3n-3	R	0.66a	0.26b	0.023	***	n.s.	*	x0.80a	0.43b	0.046	**	***	***
	C	0.61a	0.29b					y0.34	0.42				
18:4n-3	R	x0.69a	0.26b	0.025	***	**	***	x1.02a	0.22b	0084	***	***	***
	C	y0.53a	0.30b					y0.24	0.23				
20:5n-3	R	8.52b	y10.15a	0.276	***	**	***	x8.26a	y4.67b	0.525	***	n.s.	***
	C	7.79b	x12.45a					y5.78	x5.88				
22:5n-3	R	x0.98a	0.86b	0.028	*	***	**	x1.24a	0.67b	0.051	***	***	***
	C	y0.83	0.83					y0.62	0.74				
22:6n-3	R	x38.39	42.41	0.911	***	***	***	30.50	37.71	4.221	**	*	n.s.
	C	y32.74	42.71a					32.08b	47.23a				
Σ n-3PUFA[G]	R	x49.49	54.00	1.140	***	*	***	42.40	44.07	4.681	*	n.s.	*
	C	y42.71	56.70a					39.38b	54.82a				
Σ PUFA[H]	R	x55.76	57.56	1.221	**	**	***	47.96	48.18	4.778	*	n.s.	*
	C	y48.44b	60.18a					43.74b	58.99a				
Unknown	R	y8.53a	4.47b	0.207	***	*	*	11.88	5.18	1.699	***	n.s.	n.s.
	C	x9.48a	4.20b					11.85a	4.30b				
n-6/n-3	R	x0.12a	0.06b	0.002	***	n.s.	**	0.12	0.09	0.007	**	*	n.s.
	C	y0.13a	0.06b					0.10	0.07				

571

572 *Values are means of four determinations (each one in duplicate on pooled samples) per catch season*
573 *and state; means within a column and trait preceded by different letters (x, y) differ significantly ($p \leq$*
574 *0.05); means in the same row followed by different letters (a, b) differ significantly ($p \leq 0.05$); RSD =*
575 *residual standard deviation; Sc = catch season; A: includes 15:0 and 17:0; B: includes 20:1n-11, 20:1n-*
576 *9, 20:1n-7, 22:1n-11 and 22:1n-9; C: includes 18:3, 20:2, 20:3 and 22:4n-6; D: includes 16:4n-3 and*
577 *20:4n-3; E: includes 16:2n-4; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; n.s. = not significant.*

578

579 **Table 3** Content of selected fatty acids (mole%) in polar lipids from skinned fillets of sprat and horse

580 mackerel in the raw (R) and cooked (C) state (St)

Fatty acids [A,B,C]	St	**Sprat**		Statistical analysis				**Horse mackerel**		Statistical analysis			
		Winter	Spring	RSD	Sc	St	Sc*St	Autum	Spring	RSD	Sc	St	Sc*St
14:0	R	1.02	y1.31	0.074	**	**	**	0.42	0.38	0.037	n.s.	n.s.	n.s.
	C	0.97b	x1.84a					0.33	0.38				
16:0	R	22.53	22.29	1.129	n.s.	n.s.	n.s.	17.07	y16.74	1.121	n.s.	*	*
	C	22.86	21.70					17.78	x20.79				
18:0	R	3.49	x3.47	0.169	n.s.	***	**	8.51	8.67	0.558	n.s.	n.s.	n.s.
	C	3.36	y2.28					8.30	9.00				
Σ SFA[D]	R	27.96	27.95	1.182	n.s.	n.s.	n.s.	27.07	26.70	1.737	n.s.	*	n.s.
	C	28.06	26.73					27.33	31.19				
16:1n-7	R	1.44	y1.78	0.072	**	*	***	1.21a	0.77b	0.077	***	n.s.	n.s.
	C	1.27b	x2.21a					1.04a	0.75b				
18:1n-9	R	4.83	y6.25	0.170	*	*	**	x6.75	5.74	0.279	n.s.	*	*
	C	4.70	x7.05					y5.80	5.59				
18:1n-7	R	1.96	x2.18	0.094	n.s.	**	***	2.35a	1.57b	0.176	***	n.s.	n.s.
	C	2.04	y1.43					2.32a	1.56b				
20: n-9	R	0.20	y0.30	0.024	n.s.	*	n.s.						
	C	0.22	x0.38										
22:1n-11	R	0.20	y0.33	0.057	n.s.	**	**						
	C	0.20	x0.58										
Σ MUFA[E]	R	8.69	10.93	0.303	*	n.s.	*	11.00a	8.58b	0.521	**	n.s.	n.s.
	C	8.54	11.75					9.81	8.44				
18:2n-6	R	0.76	x0.72	0.018	n.s.	**	**	1.02	0.81	0.073	n.s.	n.s.	n.s.
	C	0.78	y0.61					0.92	0.80				
20:4n-6	R	1.15	1.11	0.072	*	n.s.	n.s.	3.70	2.87	0.098	*	n.s.	n.s.
	C	1.25a	1.08b					3.68	2.72				
22:5n-6	R	0.76	0.65	0.046	n.s.	n.s.	n.s.	1.97	2.17	0.128	n.s.	*	n.s.
	C	0.72	0.68					1.87	1.97				
Σ n-6 PUFA[F]	R	3.01	2.85	0.065	n.s.	n.s.	*	8.28	x7.16	0.152	*	**	n.s.
	C	3.12	2.69					7.99	y6.76				
18:3n-3	R	0.49	x0.48	0.010	n.s.	*	*	0.22	0.20	0.027	n.s.	n.s.	n.s.
	C	0.49	y0.45					0.22	0.23				
18:4n-3	R	0.50	y0.47	0.026	n.s.	**	***	0.12	0.12	0.009	n.s.	n.s.	n.s.
	C	0.48	x0.67					0.12	0.12				
20:5n-3	R	10.62	13.22	0.675	n.s.	*	n.s.	8.38	7.43	0.302	*	n.s.	n.s.
	C	11.70	14.69					8.93	7.39				
22:5n-3	R	0.70	0.70	0.032	n.s.	n.s.	n.s.	3.00	2.54	0.114	**	*	*
	C	0.67	0.72					2.97a	2.20b				
22:6n-3	R	44.46	40.21	1.661	*	n.s.	n.s.	38.02b	45.23a	2.198	**	n.s.	n.s.
	C	43.70	39.64					38.13	41.58				
Σ n-3 PUFA[G]	R	57.06	55.50	1.273	n.s.	n.s.	n.s.	49.62b	55.38a	2.490	**	n.s.	n.s.
	C	57.38	56.50					50.24	51.51				
Σ PUFA[H]	R	60.48	58.72	1.273	n.s.	n.s.	n.s.	58.23	62.83	2.585	n.s.	n.s.	n.s.
	C	60.81	59.52					58.55	58.42				

Unknown	R	2.87	x2.40	0.162	n.s.	**	n.s.	3.70a	1.89b	0.599	***	n.s.	n.s.
	C	2.59	y2.00					4.30a	1.95b				
n-6/n-3	R	0.05	0.05	0.001	n.s	n.s	n.s.	0.17a	0.13b	0.004	**	n.s.	n.s.
	C	0.05	0.05					0.16	0.13				

*Values are means of four determinations (each one in duplicate on pooled samples) per catch season and state; means within a column and trait preceded by different letters (x, y) differ significantly ($p \leq 0.05$); means in the same row followed by different letters (a, b) differ significantly ($p \leq 0.05$); RSD = residual standard deviation; Sc = catch season; A: includes 15:0 and 17:0; B: includes 20:1n-11, 20:1n-7 and 22:1n-9; C: includes 18:3, 20:2, 20:3 and 22:4n-6; D: includes 16:4n-3 and 20:4n-3; E: includes 16:2n-4; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; n.s. = not significant.*

589 **Table 4** Seasonal variation of nutritional quality indices AI (atherogenicity index), TI (thrombogenicity
590 index) and HH (hypocholesterolemic/hypercholesterolemic fatty acid ratio) in polar lipids in the raw
591 (R) and cooked (C) state (St)

Fish species	**St**	**AI**		**TI**		**HH**	
		Fall/Winter	*Spring*	*Fall/Winter*	*Spring*	*Fall/Winter*	*Spring*
Anchovy	R	y0.38±0.02	0.38±0.03	0.14±0.02	y0.14±0.02	x3.20±0.05	2.62±0.04
	C	x0.49±0.02a	0.36±0.04b	0.19±0.03	x0.25±0.04	y2.22±0.02	2.39±0.02
Sardine	R	y0.47±0.02	x0.56±0.04	0.17±0.02	x0.22±0.03	x2.46±0.04a	y1.64±0.04b
	C	x0.59±0.04a	y0.38±0.02b	0.17±0.02	y0.14±0.02	y1.75±0.03a	x2.53±0.02b
Sprat	R	0.31±0.02	0.31±0.02	0.11±0.02	0.11±0.02	3.25±0.06	2.97±0.05
	C	0.31±0.03	0.33±0.02	0.13±0.02	0.11±0.02	3.26±0.06	3.31±0.05
Horse mackerel	R	0.22±0.03	0.21±0.02	0.13±0.02	0.12±0.02	4.04±0.03	x4.49±0.05
	C	0.23±0.02	0.24±0.02	0.10±0.02	0.15±0.03	3.88±0.02	y3.38±0.06

592

593 *Values are means of four determinations ± Std Dev. Means within a column and trait preceded by*
594 *different letters (x, y) differ significantly ($p \leqslant 0.05$); means in the same row followed by different letters*
595 *(a, b) differ significantly ($p \leqslant 0.05$).*

596

597 **Table 5** Content of selected fatty acids (mole%) in neutral lipids from skin-on fillets of

598 ANCHOVY and skinned SARDINE at raw (R) and cooked (C) state.

Fatty acids [A,B,C]	St	**Anchovy**		Statistical analysis				**Sardine**		Statistical analysis			
		Fall	*Spring*	RSD	Sc	St	Sc*St	*Fall*	*Spring*	RSD	Sc	St	Sc*St
14:0	R	y6.90b	8.54a	0.257	***	n.s.	**	6.93a	4.75b	0.267	***	**	*
	C	x7.88	8.06					6.95	7.06				
16:0	R	19.39	19.68	1.316	n.s.	n.s.	n.s.	21.69	y20.89	2.078	**	n.s.	***
	C	21.62	20.24					22.96	x24.06				
18:0	R	4.97	3.91	0.334	**	*	n.s.	y5.13a	4.58b	0.451	**	*	**
	C	5.48	4.37					x6.09a	4.61b				
Σ SFA[D]	R	33.66	34.07	1.729	n.s.	*	n.s.	35.86	y32.24	2.773	n.s.	n.s.	**
	C	37.71	34.56					38.31	x38.05				
16:1n-7	R	y6.08b	9.37a	0.302	***	n.s.	**	5.50a	y3.08b	0.181	***	**	**
	C	x6.89b	9.03a					5.43	x4.06				
18:1n-9	R	y4.73	5.32	0.183	n.s.	**	n.s.	8.24	7.38	0.524	***	**	n.s.
	C	x5.20	5.63					8.71	7.84				
18:1n-7	R	2.79b	3.35a	0.116	***	*	n.s.	y2.76	2.07	0.234	n.s.	**	n.s.
	C	3.01b	3.38a					x3.27a	2.08b				
Σ MUFA[E]	R	y14.79b	19.52a	0.471	***	*	**	y18.38a	y14.49b	0.901	***	**	*
	C	x16.26b	19.16a					x19.33a	x16.42b				
18:2n-6	R	1.54a	1.18b	0.052	***	*	n.s.	1.61	1.64	0.083	*	***	**
	C	1.61a	1.23b					1.66	1.72				
20:4n-6	R	1.90a	0.90b	0.085	***	n.s.	n.s.	1.25	0.92	0.200	***	n.s.	n.s.
	C	1.74a	0.92b					1.21	0.76				
22:5n-6	R	x1.09a	0.43b	0.054	***	*	**	0.72	x0.83	0.112	n.s.	n.s.	n.s.
	C	y0.90a	0.45b					0.72	y0.64				
Σ n-6 PUFA[F]	R	5.56a	3.13b	0.215	***	n.s.	n.s.	5.44	5.00	0.216	***	**	**
	C	5.33a	3.26b					5.51a	4.71b				
18:3n-3	R	1.28	1.11	0.038	**	n.s.	n.s.	1.21	y1.26	0.046	**	***	***
	C	1.29a	1.08b					1.12	x1.47				
18:4n-3	R	1.76b	2.66a	0.392	***	n.s.	n.s.	1.68	1.78	0.084	***	***	***
	C	1.70b	2.53a					1.46	1.86				
20:5n-3	R	12.12b	15.88a	0.606	***	*	n.s.	10.35	8.19	0.525	***	n.s.	***
	C	10.64b	15.90a					9.76a	7.14b				
22:5n-3	R	x1.27a	0.80b	0.071	***	n.s.	**	1.47	x1.11	0.051	***	***	***
	C	y1.06a	0.86b					1.44a	y0.91b				
22:6n-3	R	x20.46a	13.04b	0.995	***	**	**	17.99b	x26.60a	4.221	**	*	n.s.
	C	y16.12a	13.32b					17.52	y19.51				
Σ n-3PUFA[G]	R	x37.34	33.89	1.549	n.s.	**	**	33.42	x39.59	4.681	*	n.s.	*
	C	y31.19	34.17					31.95	y31.43				
Σ PUFA[H]	R	x43.65a	37.73b	1.575	*	**	**	39.65	x45.44	4.778	*	n.s.	*
	C	y37.42	38.11					38.35	y36.92				
Unknown	R	7.74	8.36	0.007	***	*	n.s.	x6.67	8.56	0.007	**	*	n.s.
	C	8.41	8.00					y4.71b	9.20a				
n-6/n-3	R	y0.15a	0.09b	0.821	n.s.	n.s.	n.s.	0.16	y0.13	1.699	***	n.s.	n.s.
	C	x0.17a	0.10b					0.17	x0.15				

[A] Values are means of 4 determinations (each one in duplicate on pooled samples) per season of catch and state; [B] Means within a column and trait preceded by different letters (x,y) differ significantly ($P \leq 0.05$); means on the same row followed by different letters (a,b) differ significantly ($P \leq 0.05$); [C] RSD = Residual Standard Deviation; Sc = Season of catch; St = State; [D] Includes: 15:0 and 17:0; [E] Includes: 20:1n-11; 20:1n-9; 20:1n-7; 22:1n-11 and 22:1n-9; [F] Includes: 18:3, 20:2, 20:3 and 22:4n-6; [G] Includes: 16:4 and 20:4n-3; [H] Includes: 16:2n-4; *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; n.s. = not significant.

608 **Table 6** Content of selected fatty acids (mole%) in neutral lipid from skinned fillets of SPRAT

609 and HORSE MACKEREL at raw (R) and cooked (C) state.

Fatty acids [A,B,C]	St	**Sprat**		Statistical analysis				**Horse mackerel**		Statistical analysis			
		Winter	Spring	RSD	Sc	St	Sc*St	Autum	Spring	RSD	Sc	St	Sc*St
14:0	R	5.97	5.17	0.220	n.s.	n.s.	n.s.	4.16	3.31	0.157	*	n.s.	n.s.
	C	6.41	5.16					4.15	3.62				
16:0	R	25.23	22.08	0.747	n.s.	n.s.	n.s.	28.61a	20.34b	1.043	***	n.s.	*
	C	24.92	23.12					26.64a	21.53b				
18:0	R	2.97	y2.62	0.058	n.s.	**	n.s.	8.06	7.31	0.458	n.s.	n.s.	n.s.
	C	3.05	x2.81					8.02	8.14				
Σ SFA[D]	R	35.67	30.73	0.640	n.s.	n.s.	n.s.	42.76a	32.58b	1.418	***	n.s.	*
	C	35.79	32.07					40.73a	34.96b				
16:1n-7	R	7.18	6.88	0.293	n.s.	n.s.	n.s.	8.40a	4.93b	0.493	***	n.s.	n.s.
	C	7.81	6.74					8.38a	5.07b				
18:1n-9	R	16.17	16.91	0.962	n.s.	n.s.	n.s.	y16.61	15.92	0.414	n.s.	*	*
	C	17.59	17.78					x18.03	15.81				
18:1n-7	R	2.02a	y1.29b	0.078	**	**	**	y4.31	3.44	0.095	*	*	*
	C	2.07	x1.73					x4.68	3.41				
20:1 n-9	R	1.30	1.77	0.187	n.s.	n.s.	n.s.	0.39	0.74	0.051	*	n.s.	n.s.
	C	1.52	1.55					0.36	0.70				
22:1n-11	R	2.34	3.84	0.042	n.s.	n.s.	n.s.	-	0.57	-	-	-	-
	C	2.58	3.49					-	0.57				
Σ MUFA[E]	R	29.59	31.31	1.887	n.s.	n.s.	n.s.	30.49	26.26	0.819	*	n.s.	n.s.
	C	32.15	31.90					32.14	26.22				
18:2n-6	R	1.00	0.90	0.028	n.s.	n.s.	n.s.	1.08	1.28	0.267	n.s.	n.s.	n.s.
	C	1.03	0.89					1.38	1.27				
20:4n-6	R	0.44	0.36	0.061	n.s.	n.s.	n.s.	1.32	1.42	0.094	n.s.	n.s.	n.s.
	C	0.35	0.36					1.34	1.54				
22:5n-6	R	0.21	0.17	0.069	n.s.	n.s.	n.s.	0.33b	0.68a	0.049	***	n.s.	n.s.
	C	0.18	0.24					0.31b	0.59°				
Σ n-6 PUFA[F]	R	3.11a	2.58b	0.095	**	n.s.	n.s.	3.48	4.35	0.291	*	n.s.	n.s.
	C	3.06	2.64					3.93	4.35				
18:3n-3	R	1.09	x0.98	0.005	*	***	**	0.49	0.67	0.035	n.s.	n.s.	n.s.
	C	1.07	y0.94					0.53	0.66				
18:4n-3	R	2.34	2.06	0.045	n.s.	*	n.s.	0.67	0.91	0.040	n.s.	n.s.	*
	C	2.26	1.94					0.73	0.82				
20:5n-3	R	7.66	9.62	0.479	n.s.	*	n.s.	6.26	7.66	0.403	n.s.	n.s.	*
	C	6.64	9.23					6.88	6.81				
22:5n-3	R	0.41	0.65	0.030	*	n.s.	n.s.	1.24b	2.28a	0.178	**	n.s.	n.s.
	C	0.39	0.67					1.34	2.04				
22:6n-3	R	12.96	15.86	1.017	n.s.	n.s.	n.s.	7.11b	17.99a	1.513	***	n.s.	n.s.
	C	11.63	15.47					7.09b	15.77a				
Σ n-3 PUFA[G]	R	24.81	29.62	1.463	n.s.	n.s.	n.s.	15.96b	29.97a	2.106	***	n.s.	n.s.
	C	22.32	28.71					16.83b	26.46a				
Σ PUFA[H]	R	28.74	32.87	1.497	n.s.	n.s.	n.s.	20.09b	34.69a	2.198	***	n.s.	n.s.
	C	26.16	32.04					21.28b	31.18a				

Unknown	R	6.69	5.58	0.666	**	n.s.	n.s.	6.54	6.32	0.688	n.s.	n.s.	*
	C	6.42a	4.68b					5.65	7.52				
n-6/n-3	R	0.13	0.09	0.015	n.s.	n.s.	n.s.	0.22a	0.15b	0.021	**	n.s.	n.s.
	C	0.14	0.09					0.23a	0.16b				

[A] Values are means of 4 determinations (each one in duplicate on pooled samples) per season of catch and state; [B] Means within a column and trait preceded by different letters (x,y) differ significantly ($P \leq 0.05$); means on the same row followed by different letters (a,b) differ significantly ($P \leq 0.05$); [C] RSD = Residual Standard Deviation; Sc = Season of catch; St = State; [D] Includes: 15:0 and 17:0; [E] Includes: 20:1n-11; 20:1 n-7 and 22:1n-9; [F] Includes: 18:3, 20:2, 20:3 and 22:4n-6; [G] Includes: 16:4 and 20:4n-3; [H] Includes: 16: 2n-4; *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; n.s. = not significant.