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## Red muscle of small pelagic fishes' fillets are high-quality sources of essential fatty acids

--Manuscript Draft--

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| <b>Abstract:</b>              | <p>Small pelagic fishes such as sardine and anchovy are among the richest species in essential fatty acids that are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), whose bioavailability may depend on its esterification to polar or neutral lipids. The EPA and DHA quantities in neutral and polar lipids were compared in sardine (from the English Channel) and anchovy (from the Bay of Biscay) fillets, and in red and white muscle separately. Sardine fillets had the highest EPA+DHA content (<math>760\pm 670</math> vs <math>370\pm 510</math> mg/100g in anchovy fillets), mainly because of their largest proportion of lipid-rich red muscle and its relatively high lipid content. However, DHA esterified to polar lipids was higher in anchovy than in sardine fillet (<math>270\pm 60</math> vs <math>230\pm 30</math> mg/100g). EPA+DHA content were higher in red than white muscle for both species. This study highlights the nutritional interest of red muscle to provide essential dietary fatty acids to consumers, and the necessity to consider its importance in nutrition studies.</p> |
| <b>Suggested Reviewers:</b>   | Ana Gomes-Bispo, Dr<br>Portuguese Institute for the Sea and Atmosphere<br>ana.bispo@ipma.pt<br>Dr. Gomes-Bispo works on fish nutritional evaluation, especially in relation to omega-3.<br><br>Mirja Kaizer, Dr<br>University of Otago<br>mirja.ahmed@postgrad.otago.ac.nz<br>Dr. Mirja Kaizer Ahmed works on the importance of fish omega-3 for human health<br><br>Nuray Erkan<br>Dr, Department of fisheries and seafood processing<br>nurekan@istanbul.edu.tr<br>Pr. Erkan works on seafood including sardine and omega-3.<br><br>Jean-Michel Njinkoué, Dr<br>univeristy of douala<br>jmnjinkoue@yahoo.fr<br>Dr. Njinkoué works on fish lipids in red and white muscles.   |
| <b>Response to Reviewers:</b> |  |

## Red muscle of small pelagic fishes' fillets are high-quality sources of essential fatty acids

### ~~Red muscle from small pelagic fishes' fillets supply essential fatty acids~~

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## **Red muscle of small pelagic fishes' fillets are high-quality sources of essential fatty acids**

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**Highlights 3 to 5 – (85 ch incl spaces)**

- Small pelagic fish are important sources of essential dietary fatty acids
- EPA+DHA contents are higher in sardine than in anchovy fillet
- EPA+DHA contents are higher in red than in white muscle
- DHA bounded to phospholipids is higher in anchovy than in sardine fillet

# 1     **Red muscle from small pelagic fishes' fillets supply essential** 2                                   **fatty acids**

## 3 4     **Abstract**

5     Small pelagic fishes such as sardine and anchovy are among the richest species in essential  
6     fatty acids that are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), whose  
7     bioavailability may depend on its esterification to polar or neutral lipids. The EPA and DHA  
8     quantities in neutral and polar lipids were compared in sardine and anchovy fillets, and in red  
9     and white muscle separately. Sardine fillets had the highest EPA+DHA content ( $760\pm 670$  vs  
10     $370\pm 510$  mg/100g in anchovy fillets), mainly because of their largest proportion of lipid-rich  
11    red muscle and its relatively high lipid content. However, DHA esterified to polar lipids was  
12    higher in anchovy than in sardine fillet ( $270\pm 60$  vs  $230\pm 30$  mg/100g). EPA+DHA content were  
13    higher in red than white muscle for both species. This study highlights the nutritional interest  
14    of red muscle to provide essential dietary fatty acids to consumers, and the necessity to consider  
15    its importance in nutrition studies.

16    **Key words:** dark muscle, lipids, fatty acids, sardine, anchovy, biomolecule

## 17        1. Introduction

18        Omega-3 and -6 long-chain polyunsaturated fatty acids (n-3 and n-6 LC-PUFA), and especially  
19        arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic  
20        acid (DHA; 22:6n-3) are among essential dietary biomolecules involved in physiological  
21        functions in humans, such as cardiac and brain functioning, hormone synthesis, and immune  
22        response (Calder, 2018; Swanson et al., 2012). Due to the insufficient *de novo* synthesis  
23        capacities of these molecules by humans (Burdge and Calder, 2005), the World Health  
24        Organisation recommends a dietary daily intake of 250–500 mg of EPA+DHA, especially to  
25        reduce oxidative damage of the brain (Butt and Salem, 2016; FAO/WHO, 2010).

26        Bioavailability is the fraction of a nutrient in a food that is absorbed and used by an organism.  
27        It is affected by the chemical form and interactions with other food components. In the case of  
28        DHA, some recent research suggests that its chemical binding form may affect its  
29        bioavailability: a higher fraction of dietary DHA would reach the brain when esterified to  
30        (lyso)phospholipids (*i.e.*, polar lipids, that constitute cell and organelle membranes) rather than  
31        to triglycerides (*i.e.*, neutral lipids, that constitute energetic reserve) (works reviewed by  
32        Ahmmed et al., 2020; Sugasini et al., 2019). Although not systematically observed in humans  
33        (e.g., Ulven et al., 2011), such a difference could change the nutritional interest of a food.  
34        However, literature about the distribution of DHA between phospholipids and triglycerides is  
35        scarce in small pelagic fish.

36        Small pelagic fish such as European sardine (*Sardina pilchardus*, Walbaum 1792) and  
37        European anchovy (*Engraulis encrasicolus*, Linnaeus 1758) are among the richest species in  
38        EPA and DHA (*ca.* 400–1700 mg/100g). They are the largest group of species landed in marine  
39        fisheries (*ca.* a quarter of global landings; FAO, 2020), and intended to supply these molecules  
40        to the human population through direct and indirect consumptions as oils and meals for animal  
41        rearing (Gladyshev et al., 2018; Tacon and Metian, 2013). In the same way as most teleosts,  
42        small pelagic fish have two types of muscles: the red (or dark) muscle used during slow  
43        (aerobic) twitch, and the white (or light) muscle used in fast (anaerobic) twitch. Red muscle  
44        has a better ability to beta-oxidise lipids for metabolic energy than the white muscle. It is also  
45        present in a higher proportion than the white muscle in mobile fish species (McLaughlin and  
46        Kramer, 1991; Teulier et al., 2019). It constitutes a fifth to a third of the total muscular mass in  
47        pelagic fish such as Clupeidae (including anchovies and sardines) and Scombridae (Greer-  
48        Walker et al., 1975; Teulier et al., 2019). In some Clupeidae (e.g., Peruvian anchovy), the red  
49        muscle is richer in total lipids and in DHA (in g/100g) than the white muscle (Albrecht-Ruiz



50 and Salas-Maldonado, 2015). In spite of its relatively high nutritional quality, the red muscle  
51 is sometimes whitened during industrial processes (e.g. Zaghib et al., 2017) or removed before  
52 human consumption because of its distinctive taste and its poorer conservation caused by its  
53 high myoglobin and haemoglobin contents (Videler, 2011). However, in fillets of European  
54 sardine and anchovy, little is known on the contribution of the red muscle to the overall  
55 EPA+DHA content and on the proportion of DHA esterified to phospholipids.  
56 In this study, we tested the hypothesis that the content in n-3 LC-PUFA of fish fillet varies  
57 according to species (European sardine and anchovy), muscle type (red and white muscles),  
58 and lipid fraction (neutral and polar lipids). To this end, we compared fish fillets, considering  
59 the relative proportion of white and red muscles (which varies according to species), and white  
60 and red muscle separately. For all samples, we considered polar and neutral lipids separately  
61 and total lipids sum of the neutral and polar lipids).

## 62 **2. Material & Methods**

### 63 **2.1. Fish and tissues sampling**

64 Fifteen sardines *S. pilchadus* were caught during fishery surveys carried out by Ifremer in the  
65 English Channel ([CGFS](#), September 2020) and 15 anchovies *E. encrasicolus* were caught in  
66 the Bay of Biscay ([EVHOE](#), October 2020), where both species were in non-reproductive  
67 periods (Petitgas et al., 2010). Fish were dead by the time of sampling.  
68 Their fillets, containing red and white muscles, were removed and stored at -80°C on-board.  
69 Once ashore, frozen red muscle and dorsal white muscle of fillets were isolated and stored back  
70 at -80°C before subsequent analysis.

### 71 **2.2. Moisture analysis**

72 Moisture was determined by gravimetry, weighing muscle samples before and after a 72-hours  
73 freeze-drying (Christ Alpha 1-2 LD Plus lyophilizer). Immediately after freeze-drying, samples  
74 were homogenised with a manual potter and stored back at -80°C before lipid extraction.

### 75 **2.3. Lipid extraction and fatty acid analysis**

76 Lipid extraction and FA analysis were performed as described in Mathieu-Resuge et al. (2020)  
77 with slight modifications detailed below. Total lipids were extracted from *ca.* 10 mg of dry  
78 tissue with 6 mL of CHCl<sub>3</sub>:MeOH (2:1, v:v) into glass vials. The neutral and polar lipids were

79 then separated by solid phase extraction at low pressure on a silica gel micro-column with 10  
80 mL of CHCl<sub>3</sub>:MeOH (98:2, v/v) and 20 mL of MeOH, respectively. After addition of an  
81 internal standard (23:0, in free FA form), both lipid fractions were dried in an EZ-2 evaporator  
82 (Genevac). After hydrolysis in 1 ml of KOH-MeOH (0.5 M) for 30 min at 80°C, samples were  
83 transesterified with 1.6 mL of MeOH:H<sub>2</sub>SO<sub>4</sub> (3.4%; v/v) for 10 min at 100°C. FA methyl esters  
84 (FAME) were recovered in hexane and analysed on a TRACE 1300 gas chromatograph  
85 programmed in temperature and equipped with a splitless injector, a ZB-WAX column  
86 (30m×0.25mm ID×0.2µm) and a flame-ionisation detector (GC-FID, Thermo Scientific), using  
87 hydrogen as vector gas. Obtained chromatograms were processed with Chromelon 7.2 (Thermo  
88 Scientific). Fifty-five FAME were identified by comparing their retention time with references  
89 from commercial mixtures (37 components FAME, PUFA1 and PUFA3, Sigma).  
90 Quantification of FAME was based on the internal standard recovery, and then FA contents  
91 were expressed in mass fraction of wet weight (mg g<sup>-1</sup> ww and mg/100g ww) and in percentage  
92 (%) of total FA.

#### 93 **2.4. Data analysis**

94 The differences in global FA composition (in %, considering only the FA representing >1% of  
95 total FA) were analysed by permutational analysis of variance (PERMANOVA, Anderson  
96 2014) using Euclidean distances as dissimilarity values among individuals and considering  
97 species, muscle tissues and lipid fractions as factors. Principal component analyses (PCA) were  
98 performed to visualise the variation in FA profile composition between species (sardine and  
99 anchovy), muscle tissues (white and red muscles) and lipid fractions (neutral and polar lipids).  
100 The FA content of fish fillet was calculated as follows, considering the relative proportions of  
101 red and white muscle:

$$102 \text{ (Eq.1) } FA_{\text{fish fillet}} = a * FA_{\text{white muscle}} + (1-a) * FA_{\text{red muscle}}$$

103 with a=0.71 for sardine and a=0.83 for anchovy to consider the relative proportions of red and  
104 white muscles of each species (Greer- Walker et al., 1975), assuming that these values remain  
105 constant over time, and FA designated the FA of interest (either total FA, EPA+DHA or DHA  
106 contents).

107 Contents in total FA, EPA+DHA, and DHA (in mg g<sup>-1</sup>) were independently compared between  
108 species (sardine and anchovy), muscle tissues (fish fillet, white and red muscles), and lipids  
109 fraction (total, neutral and polar lipids) with Wilcoxon tests (non-parametric, with W the  
110 statistic of the test) because conditions of normality-distributed data (Shapiro-Wilks test) and

111 homoscedasticity (Bartlett test) were not respected. All statistical analyses and graphics were  
112 performed with R, with packages *Vegan* (Oksanen et al., 2020), *stats* (R Core Team, 2020) and  
113 *ggplot2* (Wickham, 2016).

114

### 115 3. Results

116 The global FA profile (%) differed between the lipid fractions (PERMANOVA,  $df=1$ ,  $r =0.82$ ,  
117  $p<0.001$ ), between the species ( $df=1$ ,  $r =0.04$ ,  $p<0.01$ ), between the lipid fractions of each  
118 species ( $df=1$ ,  $r =0.02$ ,  $p<0.01$ ), and in a lesser extent between the muscle tissues ( $df=1$ ,  
119  $r =0.003$ ,  $p<0.05$ ). The two first axes of the principal component analysis explained 57% of the  
120 total inertia (39% on the first and 18% on the second component, respectively; Fig. 1). FA  
121 profiles of neutral and polar lipids were discriminated on the first principle component, while  
122 the two species were discriminated on the second principle component (Fig. 1). Polar lipids of  
123 both species were characterised by high proportions of LC-PUFA, such as ARA, n-6 DPA  
124 (docosapentaenoic acid, 22:5n-6) and DHA, while neutral lipids contained high proportions of  
125 monounsaturated FA (MUFA) and 18C PUFA.

126 Using Eq. 1 and results provided in Table 1, we found that the total FA content of sardine  
127 fillets was on average 2.9 times higher than that of anchovy ( $37.8 \pm 15.1$  vs  $12.9 \pm 7.9$  mg g<sup>-1</sup>,  
128 respectively;  $n=15$  for each species,  $W = 9$ ,  $p < 0.01$ ; Fig. 2a). This difference was primarily  
129 due to neutral lipid FA content of fillets which was on average 3.3 times higher in sardine than  
130 in anchovy ( $25.4 \pm 14.1$  vs  $7.7 \pm 7.3$  mg g<sup>-1</sup>, respectively;  $W = 20$ ,  $p < 0.01$ , Fig. 2a), while  
131 sardine fillets' polar lipid FA content was 0.9 times lower on average than that of anchovy ( $5.1$   
132  $\pm 0.7$  vs  $5.6 \pm 1.2$  mg g<sup>-1</sup>, respectively;  $W = 157$ ,  $p < 0.05$ , Fig. 2a). The total EPA+DHA  
133 content in fish fillet was on average 2.0 times higher in sardine than in anchovy ( $7.6 \pm 6.7$  vs  
134  $3.7 \pm 5.1$  mg g<sup>-1</sup>, respectively;  $W = 1372$ ,  $p < 0.001$ ; Fig. 2b). Again, this result was primarily  
135 due to neutral lipids EPA+DHA content which was on average 3.3 times higher in sardine than  
136 in anchovy fish fillets ( $10.5 \pm 8.1$  vs  $3.2 \pm 6.3$  mg g<sup>-1</sup>, respectively;  $W =191$ ,  $p < 0.01$ ; Fig.  
137 2b) while there were no differences in polar lipids EPA+DHA contents between species ( $4.5 \pm$   
138  $2.8$  and  $4.1 \pm 3.5$  mg g<sup>-1</sup>, for sardine and anchovy respectively;  $W = 580$ ,  $p = 0.43$ ; Fig. 2b).  
139 The total DHA content in fish fillet was on average 1.4 times significantly higher and more  
140 variable in sardine than in anchovy ( $5.8 \pm 7.7$  vs  $4.1 \pm 1.6$  mg g<sup>-1</sup>, respectively;  $W = 175$ ,  $p <$   
141  $0.01$ ; Fig. 2c). The DHA content in neutral lipids of the fish fillet was on average 2.4 times  
142 higher in sardine than in anchovy, while in polar lipids it was 1.2 lower in sardine than in

143 anchovy (neutral lipids:  $3.6 \pm 1.6$  vs  $1.5 \pm 1.3$  mg g<sup>-1</sup>; W = 172, p < 0.01; polar lipids:  $2.3 \pm 0.3$   
144 vs  $2.7 \pm 0.6$  mg g<sup>-1</sup>, respectively; W = 41, p < 0.01; Fig. 2c).

145 In both species, the total FA content of the red muscle was always significantly higher  
146 than in the white muscle, whatever the lipid fraction considered (Table 1; Wilcoxon tests).  
147 Specifically, EPA+DHA content were higher in the red muscle than in the white muscle:  
148 EPA+DHA content in neutral lipids was significantly 13.6 and 4.4 times higher in the red  
149 muscle compared to the white muscle for anchovy and sardine, respectively (Table 1; W = 332,  
150 p < 0.01 and W = 222, p < 0.01, respectively). The same difference was observed in polar  
151 lipids, with 4.7 and 3.7 times significantly higher EPA+DHA content in the red muscle than in  
152 the white muscle, for anchovy and sardine, respectively (Table 1; W = 450, p < 0.01 and W =  
153 210, p < 0.01). Finally, DHA content in neutral lipids was 14.5 and 5 times higher in the red  
154 muscle than the white muscle for anchovy and sardine, respectively (Table 1; W = 336, p <  
155 0.01 and W = 224, p < 0.01), and in polar lipids it was 4.8 and 3.5 higher (Table 1; W = 450, p  
156 < 0.01 and W = 201, p < 0.01).

#### 157 4. Discussion

158 Sardine fillets have clear nutritional interests due to their high EPA+DHA contents compared  
159 to anchovy fillets ( $760 \pm 670$  vs  $370 \pm 510$  mg/100 g ww), with a large proportion of these FA  
160 bound to the neutral lipids. Contents in EPA and DHA of both lipid fractions were higher in  
161 the red than in the white muscle in both species, being a good source these essential FA. In  
162 particular, DHA esterified to polar lipids was about 4 times higher in red than in white muscle  
163 for both species, highlighting its high nutritional quality and the interest to keep it before human  
164 consumption.

#### 165 Fatty acid contents vary according to fish species and muscle types

166 In this study, sardine fillets contained about three times more of FA (or lipid) than those of  
167 anchovy. This may be explained by the higher proportion of the lipid-rich red muscle in sardine  
168 compared to anchovy. However, some environmental (e.g., season), and biotic (e.g., period of  
169 reproduction) factors can also influence the lipid content of both muscles between species. The  
170 influence of seasonality on the lipid content of fish from a given geographic area is commonly  
171 known and affected by the phenology of the species (Luzia et al., 2003; Pethybridge et al.,  
172 2014). It is also known that during the non-reproductive period (as it is the case for the studied  
173 individuals) clupeid species generally store lipids into muscles, which can then be mobilised  
174 toward gonads during the reproductive period (Brosset et al., 2015; McBride et al., 2015).

175 These seasonal differences, largely documented for the white muscle (e.g. Pethybridge et al.,  
176 2014), may also occur in the red muscle. For instance, the red muscle of the Peruvian anchovy  
177 *E. ringens* is 2-fold richer in EPA+DHA during fall than during winter (Albrecht-Ruiz and  
178 Salas-Maldonado, 2015), probably linked to its reproductive cycle. Moreover, regardless of the  
179 season, the Peruvian anchovy contained a similar amount of EPA+DHA as the European  
180 anchovy in white muscle (ca. 0.8–1 g/100g dw vs 1 g/100g dw here) and red muscle (ca. 2.4–5.3  
181 g/100g dw vs 4.6 g/100g dw here). It is thus necessary to consider the effect of seasonal changes  
182 on total lipid and EPA+DHA contents when examining the fish fillets as a source of LC-PUFA.

183

184 The muscles of the small pelagic fish were not equivalent sources of LC-PUFA. The red muscle  
185 had a greater nutritional interest than the white muscle, due to its largest amount of EPA and  
186 DHA. Similarly, in Sardinella, the red muscle is systematically fattier and richer in EPA+DHA  
187 than the white muscle: EPA+DHA content of the Madeiran sardinella *Sardinella maderensis*  
188 and of the round sardinella *S. aurita* was 925 and 445 mg/100g ww in the white muscle, and  
189 2877 and 2460 mg/100g ww in the red muscle, respectively (Njinkoué et al., 2002). However,  
190 the respective contribution of red and white muscles to EPA and DHA supply is difficult to  
191 generalize on all small pelagic fish as available data remain scarce. Red muscle also contains  
192 more polar lipids than white muscle, which has been observed in other species (e.g. tropical  
193 tuna (Sardenne et al., 2017), and attributed to a greater amount of mitochondria and a difference  
194 in cell size, but this remains to be clarified in small pelagic fish. Moreover, the red muscle is  
195 richer in other micronutrients than white muscle, in particular in iron (20-fold higher for *E.*  
196 *ringens*; Albrecht-Ruiz & Salas-Maldonado, 2015), for which deficiencies are prevalent in  
197 several human populations (Hicks et al., 2019). For all these reasons, the red muscle of sardine  
198 and anchovy seem to be of high interest for human nutrition and it would be advisable to  
199 optimise its valorisation.

200

201 Although our study focused only on lipid and FA contents, it is important to take into account  
202 other elements such as persistent organic pollutants and trace metal elements to fully address  
203 the benefit-risks balance of the consumption of these fish fillets (Noger-Huet et al., 2022;  
204 Romanić et al., 2021; Sardenne, et al., 2020). Different levels of essential micronutrients and  
205 heavy metals were observed between white and red muscles of several species but the direction  
206 of this variation was species-specific, requiring more investigation for small pelagic fishes  
207 (e.g., yellowfin tuna, Bosch et al., 2016; striped bass and northern pike, Charette et al., 2021).  
208 Moreover, studies revealing that small pelagic fishes are safe for human consumption and

209 represent a valuable source of essential FA have generally not compared independently the red  
210 and white muscles FA and pollutant contents, and are perhaps missing structural information  
211 on the origin of both FA and pollutants.

212

### 213 **Distribution of fatty acids between lipid fractions**

214 While both lipid fractions are ingested by consumers, they may not have the same degree of  
215 assimilation and it could be of interest to consider them separately when comparing the  
216 nutritional values of food sources. In this study, polar lipids had a lower quantity and diversity  
217 of FA than neutral lipids. However, they were dominated by LC-PUFA such as ARA, 22:5n-6  
218 and DHA, highlighting the high quality of their composition and supporting the nutritional  
219 interest of this fraction. In fat fish (~2% ww or 8% dw) the proportion of polar lipids (as % of  
220 total lipids) is lower than in lean fish (Rincón-Cervera et al., 2020; Sardenne et al., 2020),  
221 which may give the misleading impression that lean fish fillets are equivalent or better sources  
222 of DHA esterified to polar lipids. For instance, we found similar %DHA in polar and neutral  
223 lipids of anchovy and sardine: regardless of the muscle type, neutral lipids contained 18-19%  
224 vs 13-15% of DHA, and polar lipids 48% vs 42-46% of DHA in anchovy and sardine,  
225 respectively. Nevertheless, our quantitative results indicate that sardine fillet (fat at the sampled  
226 season here) was a slightly better source of DHA esterified to polar lipids than anchovy fillet  
227 (leaner in comparison to studied sardines). Nonetheless, further intensive investigation is  
228 required to confirm the higher beneficial health effects of DHA esterified to polar lipids for  
229 humans before any food recommendation can be drawn by nutritional institutions.

230

### 231 **Conclusion**

232 This study highlights the importance of fish red muscle as a source of essential LC-PUFA, for  
233 human nutrition. Inter-species differences were observed in LC-PUFA contents, but to compare  
234 the nutritional benefits of small pelagic fishes, other factors are likely important to consider  
235 such as the seasonal cycle and the fishing location. There is also a crucial need to provide  
236 comparable quantitative units (e.g., in mg/g or in g/100g of wet or dry weight, giving moisture  
237 in the latter case) to express FA contents to allow for reliable comparisons between studies.

238

239

## 240 **Ethical approval**

241 Fish were sampled during the scientific surveys [CGFS](#) (September 2020) and [EVHOE](#)  
242 (October 2020), under European's data collection framework (DCF) and sampling  
243 authorisation were provided by provided French national authorities (DPMA). Fish were dead  
244 by the time of sampling.

245

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371

372

## 1 **Abstract**

2 Small pelagic fishes such as sardine and anchovy are among the richest species in essential  
3 fatty acids that are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), whose  
4 bioavailability may depend on its esterification to polar or neutral lipids. The EPA and DHA  
5 quantities in neutral and polar lipids were compared in sardine (from the English Channel) and  
6 anchovy (from the Bay of Biscay) fillets, and in red and white muscle separately. Sardine fillets  
7 had the highest EPA+DHA content ( $760\pm670$  vs  $370\pm510$  mg/100g in anchovy fillets), mainly  
8 because of their largest proportion of lipid-rich red muscle and its relatively high lipid content.  
9 However, DHA esterified to polar lipids was higher in anchovy than in sardine fillet ( $270\pm60$   
10 vs  $230\pm30$  mg/100g). EPA+DHA content were higher in red than white muscle for both species.  
11 This study highlights the nutritional interest of red muscle to provide essential dietary fatty  
12 acids to consumers, and the necessity to consider its importance in nutrition studies.

13 **Key words:** dark muscle, neutral lipids, polar lipids, fatty acids, European sardine, anchovy,  
14 biomolecule

## 15        1. Introduction

16    Omega-3 and -6 long-chain polyunsaturated fatty acids (n-3 and n-6 LC-PUFA), and especially  
17    eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3) and arachidonic  
18    acid (ARA; 20:4n-6) are among essential dietary biomolecules involved in physiological  
19    functions in humans, such as cardiac and brain functioning, hormone synthesis, and immune  
20    response (Calder, 2018; Swanson et al., 2012). Due to the insufficient *de novo* synthesis  
21    capacities of these molecules by humans (Burdge and Calder, 2005), the World Health  
22    Organisation recommends a dietary daily intake of 250–500 mg of EPA+DHA, including to  
23    reduce oxidative damage of the brain (Butt and Salem, 2016; FAO/WHO, 2010).

24    Bioavailability is the degree of absorption and utilization of a nutrient contained in a food,  
25    varying depending the physiological state of the organism. It is affected by the chemical form  
26    and interactions with other food components. In the case of DHA, some recent research  
27    suggests that its chemical binding form may affect its bioavailability: a higher fraction of  
28    dietary DHA would reach the brain when esterified to (lyso)phospholipids (*i.e.*, polar lipids,  
29    that constitute cell and organelle membranes) rather than to triglycerides (*i.e.*, neutral lipids,  
30    that constitute energetic reserve) (works reviewed by Ahmmed et al., 2020; Sugasini et al.,  
31    2019). Although not systematically observed in humans (e.g., Ulven et al., 2011), such a  
32    difference could change the nutritional interest of a food. However, literature about the  
33    distribution of DHA between phospholipids and triglycerides is scarce in small pelagic fish.

34    Small pelagic fish such as European sardine (*Sardina pilchardus*, Walbaum 1792) and  
35    European anchovy (*Engraulis encrasicolus*, Linnaeus 1758) are among the richest species in  
36    EPA and DHA (*ca.* 400–1700 mg/100g) (Gladyshev et al., 2018). They are the largest group  
37    of species landed in marine fisheries (*ca.* a quarter of global landings; FAO, 2020), and  
38    intended to supply these molecules to the human population through direct and indirect  
39    consumptions as oils and meals for animal rearing (Gladyshev et al., 2018; Tacon and Metian,  
40    2013). In the same way as most teleosts, small pelagic fish have two types of muscles: the red  
41    (or dark) muscle used during slow (aerobic) swim, and the white (or light) muscle used in fast  
42    (anaerobic) swim. Red muscle has a better ability to beta-oxidise lipids for metabolic energy  
43    than the white muscle. It is also present in a higher proportion than the white muscle in mobile  
44    fish species (McLaughlin and Kramer, 1991; Teulier et al., 2019). It constitutes a fifth to a third  
45    of the total muscular mass in pelagic fish such as Clupeidae (including anchovies and sardines)  
46    and Scombridae (Greer- Walker et al., 1975; Teulier et al., 2019). In some Clupeidae (e.g.,  
47    Peruvian anchovy), the red muscle is richer in total lipids and in DHA (in g/100g) than the

48 white muscle (Albrecht-Ruiz and Salas-Maldonado, 2015). In spite of its relatively high  
49 nutritional quality, the red muscle is sometimes whitened during industrial processes (e.g.  
50 Zaghbib et al., 2017) or removed before human consumption because of its distinctive taste  
51 and its poorer conservation caused by its high myoglobin and haemoglobin contents (Videler,  
52 2011). However, in fillets of European sardine and anchovy, little is known on the contribution  
53 of the red muscle to the overall EPA+DHA content and on the proportion of DHA esterified to  
54 phospholipids.

55 In this study, we tested the hypothesis that the content in n-3 LC-PUFA of fish fillet varies  
56 according to species (European sardine and anchovy), muscle type (red and white muscles),  
57 and lipid fraction (neutral and polar lipids). To this end, we compared fish fillets, considering  
58 the relative proportion of white and red muscles (which varies according to species), and white  
59 and red muscle separately. For all samples, we considered polar and neutral lipids separately  
60 and total lipids sum of the neutral and polar lipids).

## 61 **2. Material & Methods**

### 62 **2.1. Fish and tissues sampling**

63 Fifteen sardines *S. pilchadus* were caught during fishery surveys carried out by Ifremer in the  
64 English Channel ([CGFS](#), September 2020) and 15 anchovies *E. encrasicolus* were caught in  
65 the Bay of Biscay ([EVHOE](#), October 2020), where both species were in non-reproductive  
66 periods (Petitgas et al., 2010). In the Bay of Biscay anchovy mainly spawn between May and  
67 July (Motos et al., 1996), whereas sardines from the English Channel mainly spawn in April to  
68 June and can present a second spawning period in late October (Stratoudakis et al., 2007). Fish  
69 were dead by the time of sampling.

70 Their fillets, containing red and white muscles, were removed and stored at -80°C on-board.  
71 Once ashore, frozen red muscle and dorsal white muscle of fillets were isolated and stored back  
72 at -80°C before subsequent analysis.

### 73 **2.2. Moisture analysis**

74 Moisture was determined by gravimetry, weighing muscle samples before and after a 72-hours  
75 freeze-drying period (Christ Alpha 1-2 LD Plus lyophilizer). Immediately after freeze-drying,  
76 samples were homogenised with a manual mortar and pestle and stored back at -80°C before  
77 lipid extraction.

78

### 2.3. Lipid extraction and fatty acid analysis

79 Lipid extraction and FA analysis were performed as described in Mathieu-Resuge et al. (2020)  
80 with slight modifications detailed below. Total lipids were extracted from *ca.* 10 mg of dry  
81 tissue with 6 mL of CHCl<sub>3</sub>:MeOH (2:1, v:v) into glass vials. The neutral and polar lipids were  
82 then separated by solid phase extraction (SPE) at low pressure on a silica gel micro-column  
83 with elution by 10 mL of CHCl<sub>3</sub>:MeOH (98:2, v/v) and 20 mL of MeOH, respectively. After  
84 addition of an internal standard (23:0, in free FA form), both lipid fractions were dried in an  
85 EZ-2 evaporator (Genevac). After hydrolysis in 1 ml of KOH-MeOH (0.5 M) for 30 min at  
86 80°C, samples were transesterified with 1.6 mL of MeOH:H<sub>2</sub>SO<sub>4</sub> (3.4%; v/v) for 10 min at  
87 100°C. FA methyl esters (FAME) were recovered in hexane and analysed on a TRACE 1300  
88 gas chromatograph programmed in temperature and equipped with a splitless injector, a ZB-  
89 WAX column (30m×0.25mm ID×0.2µm) and a flame-ionisation detector (GC-FID, Thermo  
90 Scientific), using hydrogen as vector gas. Obtained chromatograms were processed with  
91 Chromelon 7.2 (Thermo Scientific). Fifty-five FAME were identified by comparing their  
92 retention time with references from three commercial mixtures (37 components FAME,  
93 PUFA1 and PUFA3, Sigma), and in-house standard mixtures from marine bivalves, fish and  
94 microalgae GC-MS certified. Quantification of FAME was based on the internal standard  
95 recovery, and then FA contents were expressed in mass fraction of wet weight (mg g<sup>-1</sup> ww and  
96 mg/100g ww) and in percentage (%) of total FA. Finally, the loss of fatty acids during the  
97 separation of neutral and polar lipids was verified by quantifying fatty acids on total lipids: the  
98 recovery rate was 91.6 ± 10.0% (n=9).

99

### 2.4. Data analysis

100 The differences in the FA composition (in %, considering only the FA representing >1% of  
101 total FA) were analysed by permutational analysis of variance (PERMANOVA, Anderson  
102 2014) using Euclidean distances as dissimilarity values among individuals and considering  
103 species, muscle tissues and lipid fractions as factors. Principal component analyses (PCA) were  
104 performed to visualise the variation in FA profile composition between species (sardine and  
105 anchovy), muscle tissues (white and red muscles) and lipid fractions (neutral and polar lipids).  
106 The FA content of fish fillet was calculated as follows, considering the relative proportions of  
107 red and white muscle:

108 (Eq.1)  $FA_{\text{fish fillet}} = a * FA_{\text{white muscle}} + (1-a) * FA_{\text{red muscle}}$

109 with  $a=0.71$  for sardine and  $a=0.83$  for anchovy to consider the relative proportions of red and  
110 white muscles of each species (Greer- Walker et al., 1975), assuming that these values remain  
111 constant over time, and FA designated the FA of interest (either total FA, EPA+DHA or DHA  
112 contents).

113 Contents in total FA, EPA+DHA, and DHA (in  $\text{mg g}^{-1}$ ) were independently compared between  
114 species (sardine and anchovy), muscle tissues (fish fillet, white and red muscles), and lipids  
115 fraction (total, neutral and polar lipids) with Wilcoxon tests (non-parametric, with  $W$  the  
116 statistic of the test) because conditions of normality-distributed data (Shapiro-Wilks test) and  
117 homoscedasticity (Bartlett test) were not respected. All statistical analyses and graphics were  
118 performed with R, with packages *Vegan* (Oksanen et al., 2020), *stats* (R Core Team, 2020) and  
119 *ggplot2* (Wickham, 2016).

120

### 121 3. Results

122 The FA profile (%) differed between the lipid fractions (PERMANOVA,  $df=1$ ,  $r =0.82$ ,  
123  $p<0.001$ ), between the species ( $df=1$ ,  $r =0.04$ ,  $p<0.01$ ), between the lipid fractions of each  
124 species ( $df=1$ ,  $r =0.02$ ,  $p<0.01$ ), and to a lesser extent between the muscle tissues ( $df=1$ ,  
125  $r =0.003$ ,  $p<0.05$ ). The two first axes of the principal component analysis explained 57% of the  
126 total inertia (39% on the first and 18% on the second component, respectively; Fig. 1). FA  
127 profiles of neutral and polar lipids were discriminated on the first principle component, while  
128 the two species were discriminated on the second principle component (Fig. 1). Polar lipids of  
129 both species were characterised by high proportions of LC-PUFA, such as ARA, n-6 DPA  
130 (docosapentaenoic acid, 22:5n-6) and DHA, while neutral lipids contained high proportions of  
131 monounsaturated FA (MUFA) and C18 PUFA.

132 Using Eq. 1 and results provided in Table 1, we found that the total FA content of sardine  
133 fillets was on average 2.9 times higher than that of anchovy ( $37.8 \pm 15.1$  vs  $12.9 \pm 7.9$   $\text{mg g}^{-1}$ ,  
134 respectively;  $n=15$  for each species,  $W = 9$ ,  $p < 0.01$ ; Fig. 2a). This difference was primarily  
135 due to neutral lipid FA content of fillets which was on average 3.3 times higher in sardine than  
136 in anchovy ( $25.4 \pm 14.1$  vs  $7.7 \pm 7.3$   $\text{mg g}^{-1}$ , respectively;  $W = 20$ ,  $p < 0.01$ , Fig. 2a), while  
137 sardine fillets' polar lipid FA content was 0.9 times lower on average than that of anchovy ( $5.1$   
138  $\pm 0.7$  vs  $5.6 \pm 1.2$   $\text{mg g}^{-1}$ , respectively;  $W = 157$ ,  $p < 0.05$ , Fig. 2a). The total EPA+DHA  
139 content in fish fillet was on average 2.0 times higher in sardine than in anchovy ( $7.6 \pm 6.7$  vs  
140  $3.7 \pm 5.1$   $\text{mg g}^{-1}$ , respectively;  $W = 1372$ ,  $p < 0.001$ ; Fig. 2b). Again, this result was primarily

141 due to neutral lipids EPA+DHA content which was on average 3.3 times higher in sardine than  
142 in anchovy fish fillets ( $10.5 \pm 8.1$  vs  $3.2 \pm 6.3$  mg g<sup>-1</sup>, respectively;  $W = 191$ ,  $p < 0.01$ ; Fig.  
143 2b) while there were no differences in polar lipids EPA+DHA contents between species ( $4.5 \pm$   
144  $2.8$  and  $4.1 \pm 3.5$  mg g<sup>-1</sup>, for sardine and anchovy respectively;  $W = 580$ ,  $p = 0.43$ ; Fig. 2b).  
145 The total DHA content in fish fillet was on average 1.4 times significantly higher and more  
146 variable in sardine than in anchovy ( $5.8 \pm 7.7$  vs  $4.1 \pm 1.6$  mg g<sup>-1</sup>, respectively;  $W = 175$ ,  $p <$   
147  $0.01$ ; Fig. 2c). The DHA content in neutral lipids of the fish fillet was on average 2.4 times  
148 higher in sardine than in anchovy, while in polar lipids it was 1.2 lower in sardine than in  
149 anchovy (neutral lipids:  $3.6 \pm 1.6$  vs  $1.5 \pm 1.3$  mg g<sup>-1</sup>;  $W = 172$ ,  $p < 0.01$ ; polar lipids:  $2.3 \pm 0.3$   
150 vs  $2.7 \pm 0.6$  mg g<sup>-1</sup>, respectively;  $W = 41$ ,  $p < 0.01$ ; Fig. 2c).

151 In both species, the total FA content of the red muscle was always significantly higher  
152 than in the white muscle, whatever the lipid fraction considered (Table 1; Wilcoxon tests).  
153 Specifically, EPA+DHA content were higher in the red muscle than in the white muscle:  
154 EPA+DHA content in neutral lipids was significantly 13.6 and 4.4 times higher in the red  
155 muscle compared to the white muscle for anchovy and sardine, respectively (Table 1;  $W = 332$ ,  
156  $p < 0.01$  and  $W = 222$ ,  $p < 0.01$ , respectively). The same difference was observed in polar  
157 lipids, with 4.7 and 3.7 times significantly higher EPA+DHA content in the red muscle than in  
158 the white muscle, for anchovy and sardine, respectively (Table 1;  $W = 450$ ,  $p < 0.01$  and  $W =$   
159  $210$ ,  $p < 0.01$ ). Finally, DHA content in neutral lipids was 14.5 and 5 times higher in the red  
160 muscle than the white muscle for anchovy and sardine, respectively (Table 1;  $W = 336$ ,  $p <$   
161  $0.01$  and  $W = 224$ ,  $p < 0.01$ ), and in polar lipids it was 4.8 and 3.5 higher (Table 1;  $W = 450$ ,  $p$   
162  $< 0.01$  and  $W = 201$ ,  $p < 0.01$ ).

#### 163 4. Discussion

164 Sardine fillets have clear nutritional interests due to their high EPA+DHA contents compared  
165 to anchovy fillets ( $760 \pm 670$  vs  $370 \pm 510$  mg/100 g ww), with a large proportion of these FA  
166 as part of the neutral lipids. Contents in EPA and DHA of both lipid fractions were higher in  
167 the red than in the white muscle in both species, being a good source these essential FA. In  
168 particular, DHA esterified to polar lipids was about 4 times higher in red than in white muscle  
169 for both species, highlighting its high nutritional quality and the interest to retain it for before  
170 human consumption.

#### 171 Fatty acid contents vary according to fish species and muscle types

172 In this study, sardine fillets contained about three times more of FA (or lipid) than those of  
173 anchovy. This may be explained by the higher proportion of the lipid-rich red muscle in sardine  
174 compared to anchovy. However, some environmental (e.g., season), and biotic (e.g., period of  
175 reproduction) factors can also influence the lipid content of both muscles between species. The  
176 influence of seasonality on the lipid content of fish from a given geographic area is commonly  
177 known and affected by the phenology of the species (Luzia et al., 2003; Pethybridge et al.,  
178 2014). It is also known that during the non-reproductive period (as it is the case for the studied  
179 individuals) clupeid species generally store lipids into muscles, which can then be mobilised  
180 toward gonads during the reproductive period (Brosset et al., 2015; McBride et al., 2015).  
181 These seasonal differences, largely documented for the white muscle (e.g. Pethybridge et al.,  
182 2014), may also occur in the red muscle. For instance, the red muscle of the Peruvian anchovy  
183 *E. ringens* is 2-fold richer in EPA+DHA during fall than during winter (Albrecht-Ruiz and  
184 Salas-Maldonado, 2015), probably linked to its reproductive cycle. Moreover, regardless of the  
185 season, the Peruvian anchovy contained a similar amount of EPA+DHA as the European  
186 anchovy in white muscle (*ca.* 0.8–1 g/100g dw *vs* 1 g/100g dw here) and red muscle (*ca.* 2.4–5.3  
187 g/100g dw *vs* 4.6 g/100g dw here). It is thus necessary to consider the effect of seasonal changes  
188 on total lipid and EPA+DHA contents when examining the fish fillets as a source of LC-PUFA.  
189

190 The muscles of the small pelagic fish were not equivalent sources of LC-PUFA. The red muscle  
191 had a greater nutritional interest than the white muscle, due to its largest amount of EPA and  
192 DHA. Similarly, in sardinella, the red muscle is systematically fattier and richer in EPA+DHA  
193 than the white muscle: EPA+DHA content of the Madeiran sardinella *Sardinella maderensis*  
194 and of the round sardinella *S. aurita* was 925 and 445 mg/100g ww in the white muscle, and  
195 2877 and 2460 mg/100g ww in the red muscle, respectively (Njinkoué et al., 2002). However,  
196 the respective contribution of red and white muscles to EPA and DHA supply is difficult to  
197 generalize for all small pelagic fish as available data remain scarce, as well as information on  
198 the influence of seasonal, spatial and nutritional conditions on variations in the proportions of  
199 red and white muscles. Red muscle also contains more polar lipids than white muscle, which  
200 has been observed in other species (e.g. tropical tuna (Sardenne et al., 2017), and attributed to  
201 a greater amount of mitochondria and a difference in cell size, but this remains to be clarified  
202 in small pelagic fish. Moreover, the red muscle is richer in other micronutrients than white  
203 muscle, in particular in iron (20-fold higher for *E. ringens*; Albrecht-Ruiz & Salas-Maldonado,  
204 2015), for which deficiencies are prevalent in several human populations (Hicks et al., 2019).



205 For all these reasons, the red muscle of sardine and anchovy seem to be of high interest for  
206 human nutrition and it would be advisable to optimise its valorisation.

207

208 Although our study focused only on lipid and FA contents, it is important to take into account  
209 other elements such as persistent organic pollutants and trace metal elements to fully address  
210 the benefit-risks balance of the consumption of these fish fillets (Noger-Huet et al., 2022;  
211 Romanić et al., 2021; Sardenne, et al., 2020). Different levels of essential micronutrients and  
212 heavy metals were observed between white and red muscles of several species but the direction  
213 of this variation was species-specific, requiring more investigation for small pelagic fishes  
214 (e.g., yellowfin tuna, Bosch et al., 2016; striped bass and northern pike, Charette et al., 2021).  
215 Moreover, studies revealing that small pelagic fishes are safe for human consumption and  
216 represent a valuable source of essential FA have generally not compared independently the red  
217 and white muscles FA and pollutant contents, and are perhaps missing structural information  
218 on the origin of both FA and pollutants.

219

#### 220 **Distribution of fatty acids between lipid fractions**

221 While both lipid fractions are ingested by consumers, they may not have the same degree of  
222 assimilation and it could be of interest to consider them separately when comparing the  
223 nutritional values of food sources. In this study, polar lipids had a lower quantity and diversity  
224 of FA than neutral lipids. However, they were dominated by LC-PUFA such as ARA, 22:5n-6  
225 and DHA, highlighting the high quality of their composition and supporting the nutritional  
226 interest of this fraction. In fat fish (~2% ww or 8% dw) the proportion of polar lipids (as % of  
227 total lipids) is lower than in lean fish (Rincón-Cervera et al., 2020; Sardenne et al., 2020),  
228 which may give the misleading impression that lean fish fillets are equivalent or better sources  
229 of DHA esterified to polar lipids. For instance, we found similar %DHA in polar and neutral  
230 lipids of anchovy and sardine: regardless of the muscle type, neutral lipids contained 18-19%  
231 vs 13-15% of DHA, and polar lipids 48% vs 42-46% of DHA in anchovy and sardine,  
232 respectively. Nevertheless, our quantitative results indicate that sardine fillet (fat at the sampled  
233 season here) was a slightly better source of DHA esterified to polar lipids than anchovy fillet  
234 (leaner in comparison to studied sardines). Nonetheless, further intensive investigation is  
235 required to confirm the higher beneficial health effects of DHA esterified to polar lipids for  
236 humans before any food recommendation can be drawn by nutritional institutions.

237

#### 238 **Conclusion**

239 This study highlights the importance of the red muscle of European sardine and anchovy as a  
240 source of essential LC-PUFA, for human nutrition. Inter-species differences were observed in  
241 LC-PUFA contents, but to compare the nutritional benefits of small pelagic fishes, other factors  
242 are likely important to consider such as the seasonal cycle and the fishing location. There is  
243 also a crucial need to provide comparable quantitative units (e.g., in mg/g or in g/100g of wet  
244 or dry weight, giving moisture in the latter case) to express FA contents to allow for reliable  
245 comparisons between studies, as already highlighted by Gladyshev et al., (2018).

## 246 **Ethical approval**

247 Fish were sampled during the scientific surveys [CGFS](#) (September 2020) and [EVHOE](#)  
248 (October 2020), under European's data collection framework (DCF) and sampling  
249 authorisation were provided by provided French national authorities (DPMA). Fish were dead  
250 by the time of sampling.

251

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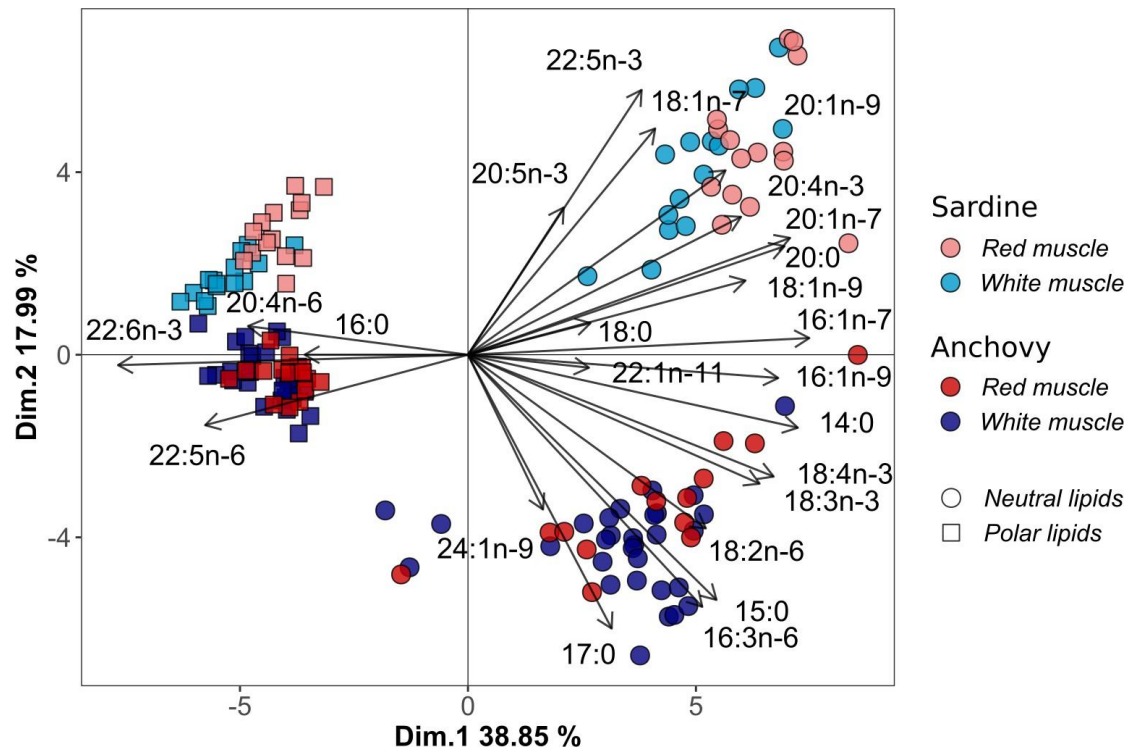
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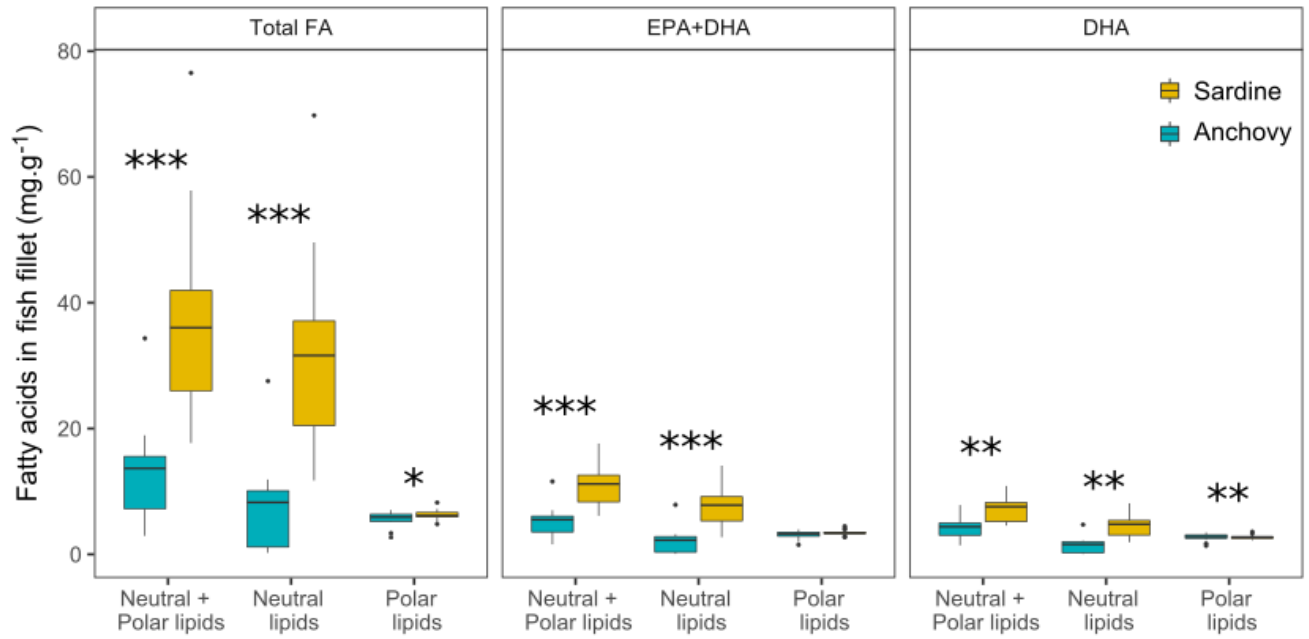
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2

3 **Figure 1.** Principal component analysis (PCA) of the fatty acid composition (mass %) of  
 4 neutral and polar lipids (displayed by dot shape), in red and white muscles (displayed by  
 5 colours) of both species (sardine *S. pilchardus* and anchovy *E. encrasicolus*, displayed by  
 6 colour intensity).

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**Figure 2.** Total fatty acid, EPA+DHA and DHA contents (mg g<sup>-1</sup>) in total, neutral and polar lipids of sardine *S. pilchardus* and anchovy *E. encrasicolus* fillets (*i.e.* considering the relative proportions of white and red muscles in the fillets). Significant inter-specific differences are indicated with stars (Wilcoxon tests; \* =p <0.05, \*\* =p <0.01, \*\*\* =p <0.001).

- 1 **Table 1.** Fatty acid compositions of neutral and polar lipid fractions (mean  $\pm$  SD; mg g<sup>-1</sup> wet weight) in red and white muscles of anchovy *E.*  
 2 *encrasicolus* and sardine *S. pilchardus*. Capital letters indicate significant differences ( $p < 0.05$ ) within polar lipids and minuscule letters indicate  
 3 significant differences within neutral lipids, between both muscles for each species independently (Wilcoxon tests).

| Fatty acids   | Anchovy                      |                            |                            |                            | Sardine                     |                            |                            |                            |
|---------------|------------------------------|----------------------------|----------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|
|               | Red muscle                   |                            | White muscle               |                            | Red muscle                  |                            | White muscle               |                            |
|               | Neutral lipids               | Polar lipids               | Neutral lipids             | Polar lipids               | Neutral lipids              | Polar lipids               | Neutral lipids             | Polar lipids               |
| ARA           | 0.3 $\pm$ 0.4 <sup>a</sup>   | 0.2 $\pm$ 0.1 <sup>A</sup> | 0.0 $\pm$ 0.0 <sup>b</sup> | 0.0 $\pm$ 0.0 <sup>B</sup> | 0.7 $\pm$ 0.2 <sup>a</sup>  | 0.2 $\pm$ 0.0 <sup>A</sup> | 0.1 $\pm$ 0.1 <sup>b</sup> | 0.1 $\pm$ 0.0 <sup>B</sup> |
| EPA           | 2.9 $\pm$ 3.6 <sup>a</sup>   | 1.0 $\pm$ 0.3 <sup>A</sup> | 0.2 $\pm$ 0.2 <sup>b</sup> | 0.3 $\pm$ 0.1 <sup>B</sup> | 6.7 $\pm$ 2.5 <sup>a</sup>  | 1.6 $\pm$ 0.6 <sup>A</sup> | 1.7 $\pm$ 1.5 <sup>b</sup> | 0.3 $\pm$ 0.1 <sup>B</sup> |
| DHA           | 5.8 $\pm$ 5.7 <sup>a</sup>   | 7.6 $\pm$ 1.9 <sup>A</sup> | 0.4 $\pm$ 0.4 <sup>b</sup> | 1.6 $\pm$ 0.4 <sup>B</sup> | 10.5 $\pm$ 3.6 <sup>a</sup> | 5.6 $\pm$ 0.9 <sup>A</sup> | 2.1 $\pm$ 1.6 <sup>b</sup> | 1.6 $\pm$ 0.3 <sup>B</sup> |
| EPA+DHA       | 8.7 $\pm$ 9.2 <sup>a</sup>   | 8.7 $\pm$ 2.2 <sup>A</sup> | 0.6 $\pm$ 0.5 <sup>b</sup> | 1.8 $\pm$ 0.4 <sup>B</sup> | 17.2 $\pm$ 5.6 <sup>a</sup> | 7.3 $\pm$ 1.2 <sup>A</sup> | 3.9 $\pm$ 3.0 <sup>b</sup> | 1.9 $\pm$ 0.3 <sup>B</sup> |
| $\Sigma$ n-3  | 10.7 $\pm$ 11.6 <sup>a</sup> | 9.1 $\pm$ 2.3 <sup>A</sup> | 0.8 $\pm$ 0.7 <sup>b</sup> | 1.9 $\pm$ 0.5 <sup>B</sup> | 21.6 $\pm$ 7.3 <sup>a</sup> | 7.6 $\pm$ 1.3 <sup>A</sup> | 4.9 $\pm$ 3.9 <sup>b</sup> | 2.0 $\pm$ 0.3 <sup>B</sup> |
| $\Sigma$ n-6  | 1.5 $\pm$ 1.7 <sup>a</sup>   | 0.7 $\pm$ 0.2 <sup>A</sup> | 0.1 $\pm$ 0.1 <sup>b</sup> | 0.1 $\pm$ 0.0 <sup>B</sup> | 2.6 $\pm$ 0.8 <sup>a</sup>  | 0.5 $\pm$ 0.1 <sup>A</sup> | 0.5 $\pm$ 0.4 <sup>b</sup> | 0.1 $\pm$ 0.0 <sup>B</sup> |
| n-3/n-6       | 7.1 <sup>a</sup>             | 13.2 <sup>A</sup>          | 8.0 <sup>b</sup>           | 17.1 <sup>B</sup>          | 8.3 <sup>a</sup>            | 16.3 <sup>A</sup>          | 9.9 <sup>b</sup>           | 18.7 <sup>B</sup>          |
| $\Sigma$ SFA  | 12.0 $\pm$ 12.3 <sup>a</sup> | 4.7 $\pm$ 1.2 <sup>A</sup> | 0.8 $\pm$ 0.7 <sup>b</sup> | 1.1 $\pm$ 0.3 <sup>B</sup> | 24.5 $\pm$ 8.7 <sup>a</sup> | 4.0 $\pm$ 0.7 <sup>A</sup> | 5.7 $\pm$ 4.8 <sup>b</sup> | 1.1 $\pm$ 0.2 <sup>B</sup> |
| $\Sigma$ MUFA | 6.8 $\pm$ 6.9 <sup>a</sup>   | 1.3 $\pm$ 0.4 <sup>A</sup> | 0.4 $\pm$ 0.4 <sup>b</sup> | 0.2 $\pm$ 0.1 <sup>B</sup> | 20.6 $\pm$ 8.6 <sup>a</sup> | 1.0 $\pm$ 0.2 <sup>A</sup> | 4.5 $\pm$ 4.3 <sup>b</sup> | 0.2 $\pm$ 0.1 <sup>B</sup> |
| $\Sigma$ PUFA | 12.6 $\pm$ 14.0 <sup>a</sup> | 9.8 $\pm$ 2.5 <sup>A</sup> | 0.9 $\pm$ 0.8 <sup>b</sup> | 2.0 $\pm$ 0.5 <sup>B</sup> | 25.2 $\pm$ 8.4 <sup>a</sup> | 8.1 $\pm$ 1.4 <sup>A</sup> | 5.7 $\pm$ 4.5 <sup>b</sup> | 2.1 $\pm$ 0.3 <sup>B</sup> |



|                  |                          |                         |                        |                        |                          |                         |                          |                        |
|------------------|--------------------------|-------------------------|------------------------|------------------------|--------------------------|-------------------------|--------------------------|------------------------|
| <b>Σ LC-PUFA</b> | 9.9 ± 10.6 <sup>a</sup>  | 9.4 ± 2.4 <sup>A</sup>  | 0.7 ± 0.6 <sup>b</sup> | 2.0 ± 0.5 <sup>B</sup> | 20.8 ± 6.8 <sup>a</sup>  | 7.9 ± 1.3 <sup>A</sup>  | 4.7 ± 3.7 <sup>b</sup>   | 2.1 ± 0.3 <sup>B</sup> |
| <b>Total FA</b>  | 31.7 ± 33.1 <sup>a</sup> | 15.9 ± 4.0 <sup>A</sup> | 2.1 ± 1.8 <sup>b</sup> | 3.3 ± 0.8 <sup>B</sup> | 70.7 ± 24.5 <sup>a</sup> | 13.2 ± 2.3 <sup>A</sup> | 15.9 ± 13.7 <sup>b</sup> | 3.5 ± 0.5 <sup>b</sup> |

*EPA, Eicosapentaenoic acid (20:5n-3); DHA, Docosahexaenoic acid (22:6n-3); SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; LC-PUFA, long chain polyunsaturated FA ≥ 20C (n=14 fatty acids)*

**Supplementary table 1-** Fatty acid compositions of neutral and polar lipid fractions (mean  $\pm$  SD; mg g<sup>-1</sup> wet weight) in red and white muscles of anchovy *E. encrasicolus* and sardine *S. pilchardus*. Only the fatty acids accounting for >1% of the total fatty acids in at least one sample are shown.

|                  | Anchovy                           |                                |                                 |                                 | Sardine                           |                                  |                                   |                                 |
|------------------|-----------------------------------|--------------------------------|---------------------------------|---------------------------------|-----------------------------------|----------------------------------|-----------------------------------|---------------------------------|
|                  | Red muscle                        |                                | White muscle                    |                                 | Red muscle                        |                                  | White muscle                      |                                 |
|                  | Neutral lipids                    | Polar lipids                   | Neutral lipids                  | Polar lipids                    | Neutral lipids                    | Polar lipids                     | Neutral lipids                    | Polar lipids                    |
| 14:0             | 2.3 $\pm$ 3                       | 0.1 $\pm$ 0                    | 0.1 $\pm$ 0.2                   | 0 $\pm$ 0                       | 3.2 $\pm$ 1.2                     | 0.1 $\pm$ 0                      | 0.7 $\pm$ 0.6                     | 0 $\pm$ 0                       |
| 15:0             | 0.4 $\pm$ 0.4                     | 0.1 $\pm$ 0                    | 0 $\pm$ 0                       | 0 $\pm$ 0                       | 0.5 $\pm$ 0.2                     | 0 $\pm$ 0                        | 0.1 $\pm$ 0.1                     | 0 $\pm$ 0                       |
| 16:0             | 7.2 $\pm$ 7                       | 3.4 $\pm$ 0.9                  | 0.5 $\pm$ 0.4                   | 0.8 $\pm$ 0.2                   | 15.2 $\pm$ 6.4                    | 3.2 $\pm$ 0.5                    | 3.5 $\pm$ 3                       | 0.9 $\pm$ 0.1                   |
| 17:0             | 0.3 $\pm$ 0.3                     | 0.2 $\pm$ 0                    | 0 $\pm$ 0                       | 0 $\pm$ 0                       | 0.5 $\pm$ 0.1                     | 0.1 $\pm$ 0                      | 0.1 $\pm$ 0.1                     | 0 $\pm$ 0                       |
| 18:0             | 1.4 $\pm$ 1.4                     | 0.9 $\pm$ 0.2                  | 0.1 $\pm$ 0.1                   | 0.1 $\pm$ 0                     | 4.3 $\pm$ 2.1                     | 0.6 $\pm$ 0.1                    | 1 $\pm$ 0.9                       | 0.2 $\pm$ 0                     |
| $\Sigma$ SFA     | 12 $\pm$ 12.3                     | 4.7 $\pm$ 1.2                  | 0.8 $\pm$ 0.7                   | 1.1 $\pm$ 0.3                   | 24.5 $\pm$ 8.7                    | 4 $\pm$ 0.7                      | 5.7 $\pm$ 4.8                     | 1.1 $\pm$ 0.2                   |
| 16:1n-7          | 1.8 $\pm$ 2.7                     | 0.1 $\pm$ 0.1                  | 0.1 $\pm$ 0.1                   | 0 $\pm$ 0                       | 3.5 $\pm$ 1.4                     | 0.1 $\pm$ 0                      | 0.8 $\pm$ 0.8                     | 0 $\pm$ 0                       |
| 18:1n-7          | 0.6 $\pm$ 0.7                     | 0.3 $\pm$ 0.1                  | 0 $\pm$ 0                       | 0.1 $\pm$ 0                     | 2.5 $\pm$ 0.9                     | 0.2 $\pm$ 0.1                    | 0.5 $\pm$ 0.5                     | 0.1 $\pm$ 0                     |
| 18:1n-9          | 3 $\pm$ 2.9                       | 0.7 $\pm$ 0.2                  | 0.2 $\pm$ 0.2                   | 0.1 $\pm$ 0                     | 9.5 $\pm$ 5.1                     | 0.4 $\pm$ 0.2                    | 2.2 $\pm$ 2.4                     | 0.1 $\pm$ 0                     |
| 20:1n-9          | 0.3 $\pm$ 0.4                     | 0 $\pm$ 0                      | 0 $\pm$ 0                       | 0 $\pm$ 0                       | 1.8 $\pm$ 0.9                     | 0.1 $\pm$ 0                      | 0.4 $\pm$ 0.4                     | 0 $\pm$ 0                       |
| 22:1n-11         | 0.2 $\pm$ 0.6                     | 0 $\pm$ 0                      | 0 $\pm$ 0                       | 0 $\pm$ 0                       | 0.5 $\pm$ 1.1                     | 0 $\pm$ 0                        | 0.1 $\pm$ 0.1                     | 0 $\pm$ 0                       |
| 24:1n-9          | 0.3 $\pm$ 0.2                     | 0 $\pm$ 0                      | 0 $\pm$ 0                       | 0 $\pm$ 0                       | 0.8 $\pm$ 0.3                     | 0 $\pm$ 0                        | 0.1 $\pm$ 0.1                     | 0 $\pm$ 0                       |
| $\Sigma$ MUFA    | 6.8 $\pm$ 6.9                     | 1.3 $\pm$ 0.4                  | 0.4 $\pm$ 0.4                   | 0.2 $\pm$ 0.1                   | 20.6 $\pm$ 8.6                    | 1 $\pm$ 0.2                      | 4.5 $\pm$ 4.3                     | 0.2 $\pm$ 0.1                   |
| 16:4n-1          | 0.1 $\pm$ 0.2                     | 0 $\pm$ 0                      | 0 $\pm$ 0                       | 0 $\pm$ 0                       | 0.2 $\pm$ 0.2                     | 0 $\pm$ 0                        | 0.1 $\pm$ 0.1                     | 0 $\pm$ 0                       |
| 18:2n-6          | 0.5 $\pm$ 0.5                     | 0.2 $\pm$ 0                    | 0 $\pm$ 0                       | 0 $\pm$ 0                       | 0.6 $\pm$ 0.3                     | 0.1 $\pm$ 0                      | 0.1 $\pm$ 0.1                     | 0 $\pm$ 0                       |
| 18:3n-3          | 0.4 $\pm$ 0.5                     | 0.1 $\pm$ 0                    | 0 $\pm$ 0                       | 0 $\pm$ 0                       | 0.7 $\pm$ 0.3                     | 0 $\pm$ 0                        | 0.2 $\pm$ 0.1                     | 0 $\pm$ 0                       |
| 18:4n-3          | 0.8 $\pm$ 1.1                     | 0 $\pm$ 0                      | 0.1 $\pm$ 0.1                   | 0 $\pm$ 0                       | 1.2 $\pm$ 0.6                     | 0 $\pm$ 0                        | 0.3 $\pm$ 0.3                     | 0 $\pm$ 0                       |
| 20:4n-3          | 0.1 $\pm$ 0.2                     | 0 $\pm$ 0                      | 0 $\pm$ 0                       | 0 $\pm$ 0                       | 0.6 $\pm$ 0.2                     | 0.1 $\pm$ 0                      | 0.1 $\pm$ 0.1                     | 0 $\pm$ 0                       |
| 20:4n-6          | 0.3 $\pm$ 0.4                     | 0.2 $\pm$ 0.1                  | 0 $\pm$ 0                       | 0 $\pm$ 0                       | 0.7 $\pm$ 0.2                     | 0.2 $\pm$ 0                      | 0.1 $\pm$ 0.1                     | 0.1 $\pm$ 0                     |
| 20:5n-3          | 2.9 $\pm$ 3.6                     | 1 $\pm$ 0.3                    | 0.2 $\pm$ 0.2                   | 0.3 $\pm$ 0.1                   | 6.7 $\pm$ 2.5                     | 1.6 $\pm$ 0.6                    | 1.7 $\pm$ 1.5                     | 0.3 $\pm$ 0.1                   |
| 22:5n-3          | 0.2 $\pm$ 0.2                     | 0.1 $\pm$ 0                    | 0 $\pm$ 0                       | 0 $\pm$ 0                       | 1.1 $\pm$ 0.4                     | 0.2 $\pm$ 0                      | 0.3 $\pm$ 0.2                     | 0 $\pm$ 0                       |
| 22:5n-6          | 0.2 $\pm$ 0.2                     | 0.2 $\pm$ 0                    | 0 $\pm$ 0                       | 0 $\pm$ 0                       | 0.3 $\pm$ 0.1                     | 0.1 $\pm$ 0                      | 0.1 $\pm$ 0                       | 0 $\pm$ 0                       |
| 22:6n-3          | 5.8 $\pm$ 5.7                     | 7.6 $\pm$ 1.9                  | 0.4 $\pm$ 0.4                   | 1.6 $\pm$ 0.4                   | 10.5 $\pm$ 3.6                    | 5.6 $\pm$ 0.9                    | 2.1 $\pm$ 1.6                     | 1.6 $\pm$ 0.3                   |
| $\Sigma$ PUFA    | 12.6 $\pm$ 14                     | 9.8 $\pm$ 2.5                  | 0.9 $\pm$ 0.8                   | 2 $\pm$ 0.5                     | 25.2 $\pm$ 8.4                    | 8.1 $\pm$ 1.4                    | 5.7 $\pm$ 4.5                     | 2.1 $\pm$ 0.3                   |
| $\Sigma$ LC-PUFA | 9.9 $\pm$ 10.6                    | 9.4 $\pm$ 2.4                  | 0.7 $\pm$ 0.6                   | 2 $\pm$ 0.5                     | 20.8 $\pm$ 6.8                    | 7.9 $\pm$ 1.3                    | 4.7 $\pm$ 3.7                     | 2.1 $\pm$ 0.3                   |
| <b>Total FA</b>  | <b>31.7 <math>\pm</math> 33.1</b> | <b>15.9 <math>\pm</math> 4</b> | <b>2.1 <math>\pm</math> 1.8</b> | <b>3.3 <math>\pm</math> 0.8</b> | <b>70.7 <math>\pm</math> 24.5</b> | <b>13.2 <math>\pm</math> 2.3</b> | <b>15.9 <math>\pm</math> 13.7</b> | <b>3.5 <math>\pm</math> 0.5</b> |

SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; LC-PUFA, long chain polyunsaturated FA  $\geq$  20C (n=14 fatty acids)

**Author statement**

Margaux Mathieu-Resuge: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing original draft

Fabienne Le Grand: Data curation, Formal analysis, Methodology, Validation, Writing review

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### **Conflict of interest statement**

Authors declare to have no conflict of interest.

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