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

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| Corresponding Author: | Margaux Mathieu-Resuge Laboratoire des Sciences de l'Environnement Marin Plouzane, Bretagne FRANCE |
| First Author: | Margaux Mathieu-Resuge |
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| Abstract: | <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 (760±670 vs 370±510 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 (270±60 vs 230±30 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> |
| Suggested Reviewers: | Ana Gomes-Bispo, Dr Portuguese Institute for the Sea and Atmosphere ana.bispo@ipma.pt Dr. Gomes-Bispo works on fish nutritional evaluation, especially in relation to omega-3. Mirja Kaizer, Dr University of Otago mirja.ahmed@postgrad.otago.ac.nz Dr. Mirja Kaizer Ahmed works on the importance of fish omega-3 for human health Nuray Erkan Dr, Department of fisheries and seafood processing nurekan@istanbul.edu.tr Pr. Erkan works on seafood including sardine and omega-3. Jean-Michel Njinkoué, Dr univeristy of douala jmnjinkoue@yahoo.fr Dr. Njinkoué works on fish lipids in red and white muscles. |
| Response to Reviewers: | |

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~~

Margaux MATHIEU-RESUGE^{1,2*}, Fabienne LE GRAND¹, Pablo BROSSET³, Christophe LEBIGRE², Philippe SOUDANT¹, Marie VAGNER¹, Laure PECQUERIE¹, Fany SARDENNE¹

¹University of Brest, CNRS, IRD, Ifremer, LEMAR, F-29280, Plouzane, France

²UMR DECOD (Ecosystem Dynamics and Sustainability), Ifremer, INRAE, Institut Agro, Plouzané, France

³UMR DECOD (Ecosystem Dynamics and Sustainability), Institut Agro, Ifremer, INRAE, Rennes, France.

* Corresponding authors : Margaux Mathieu-Resuge: m.mathieuresuge@gmail.com

Fabienne Le Grand: fabienne.legrand@univ-brest.fr

Pablo Brosset: pablo.brosset@agrocampus-ouest.fr

Christophe Lebigre: christophe.lebigre@ifremer.fr

Philippe Soudant: soudant@univ-brest.fr

Marie Vagner: marie.vagner@univ-brest.fr

Laure Pecquerie: Laure.Pecquerie@ird.fr

Fany Sardenne: fany.sardenne@ird.fr

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¹University of Brest, CNRS, IRD, Ifremer, LEMAR, F-29280, Plouzane, France

²UMR DECOD (Ecosystem Dynamics and Sustainability), Ifremer, INRAE, Institut Agro, Plouzané, France

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* Corresponding authors : Margaux Mathieu-Resuge: m.mathieuresuge@gmail.com

Fabienne Le Grand: fabienne.legrand@univ-brest.fr

Pablo Brosset: pablo.brosset@agrocampus-ouest.fr

Christophe Lebigre: christophe.lebigre@ifremer.fr

Philippe Soudant: soudant@univ-brest.fr

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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

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

Abstract

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 and anchovy fillets, and in red and white muscle separately. Sardine fillets had the highest EPA+DHA content (760 ± 670 vs 370 ± 510 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 (270 ± 60 vs 230 ± 30 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.

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 CHCl₃: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 CHCl₃: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:H₂SO₄ (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⁻¹ 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⁻¹) 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 ± 15.1 vs 12.9 ± 7.9 mg g⁻¹,
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 ± 14.1 vs 7.7 ± 7.3 mg g⁻¹, 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 ± 0.7 vs 5.6 ± 1.2 mg g⁻¹, 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 ± 6.7 vs
134 3.7 ± 5.1 mg g⁻¹, 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 ± 8.1 vs 3.2 ± 6.3 mg g⁻¹, 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 ± 3.5 mg g⁻¹, 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 ± 7.7 vs 4.1 ± 1.6 mg g⁻¹, 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 ± 1.6 vs 1.5 ± 1.3 mg g⁻¹; W = 172, p < 0.01; polar lipids: 2.3 ± 0.3
144 vs 2.7 ± 0.6 mg g⁻¹, 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 ± 670 vs 370 ± 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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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 ± 670 vs 370 ± 510 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 ± 60
10 vs 230 ± 30 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 CHCl₃: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 CHCl₃: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:H₂SO₄ (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⁻¹ 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 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 ± 15.1 vs 12.9 ± 7.9 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 ± 14.1 vs 7.7 ± 7.3 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 ± 0.7 vs 5.6 ± 1.2 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 ± 6.7 vs
140 3.7 ± 5.1 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 ± 8.1 vs 3.2 ± 6.3 mg g⁻¹, 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 ± 3.5 mg g⁻¹, 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 ± 7.7 vs 4.1 ± 1.6 mg g⁻¹, 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 ± 1.6 vs 1.5 ± 1.3 mg g⁻¹; $W = 172$, $p < 0.01$; polar lipids: 2.3 ± 0.3
150 vs 2.7 ± 0.6 mg g⁻¹, 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 ± 670 vs 370 ± 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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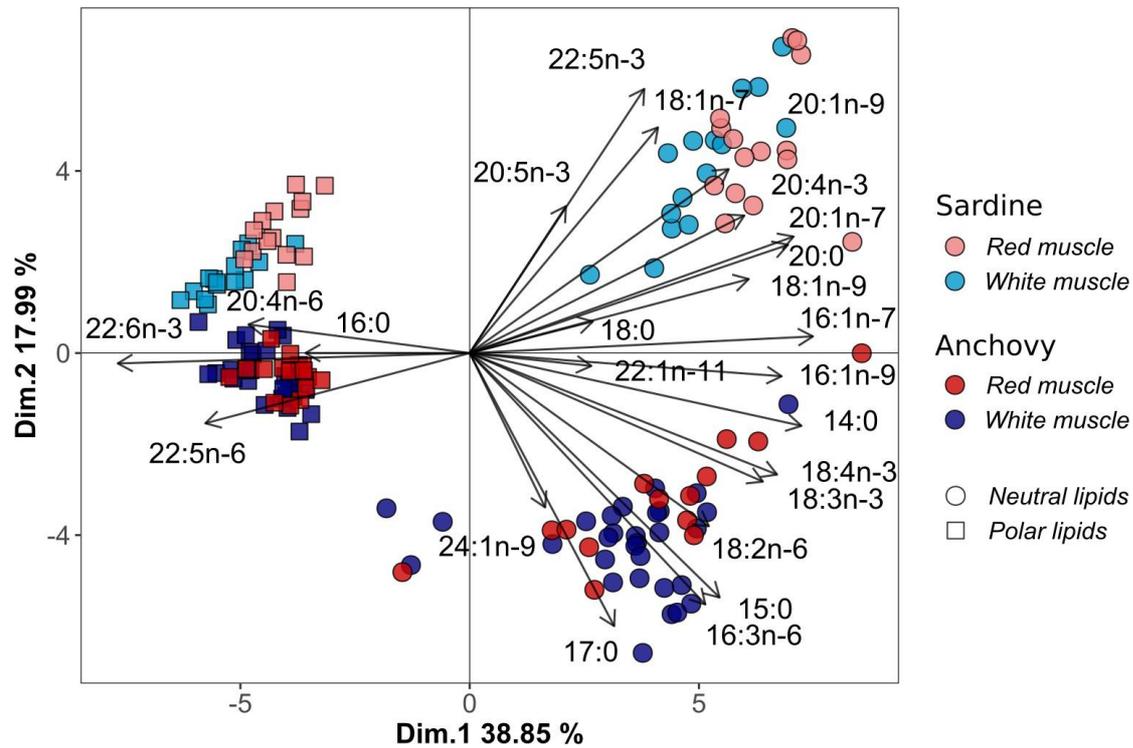
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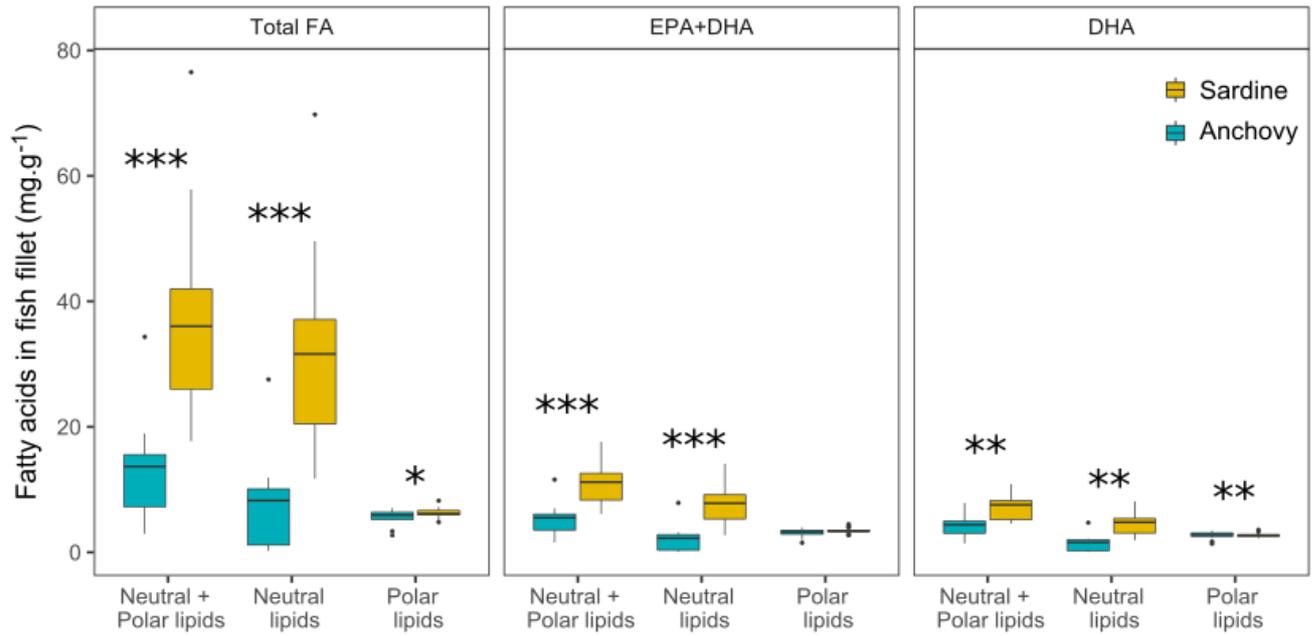
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3 **Figure 1.** Principal component analysis (PCA) of the fatty acid composition (mass %) of
 4 neutral and polar lipids (displayed by dot shape), in red and white muscles (displayed by
 5 colours) of both species (sardine *S. pilchardus* and anchovy *E. encrasicolus*, displayed by
 6 colour intensity).

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Figure 2. Total fatty acid, EPA+DHA and DHA contents (mg g⁻¹) 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⁻¹ 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 ^a | 0.2 \pm 0.1 ^A | 0.0 \pm 0.0 ^b | 0.0 \pm 0.0 ^B | 0.7 \pm 0.2 ^a | 0.2 \pm 0.0 ^A | 0.1 \pm 0.1 ^b | 0.1 \pm 0.0 ^B |
| EPA | 2.9 \pm 3.6 ^a | 1.0 \pm 0.3 ^A | 0.2 \pm 0.2 ^b | 0.3 \pm 0.1 ^B | 6.7 \pm 2.5 ^a | 1.6 \pm 0.6 ^A | 1.7 \pm 1.5 ^b | 0.3 \pm 0.1 ^B |
| DHA | 5.8 \pm 5.7 ^a | 7.6 \pm 1.9 ^A | 0.4 \pm 0.4 ^b | 1.6 \pm 0.4 ^B | 10.5 \pm 3.6 ^a | 5.6 \pm 0.9 ^A | 2.1 \pm 1.6 ^b | 1.6 \pm 0.3 ^B |
| EPA+DHA | 8.7 \pm 9.2 ^a | 8.7 \pm 2.2 ^A | 0.6 \pm 0.5 ^b | 1.8 \pm 0.4 ^B | 17.2 \pm 5.6 ^a | 7.3 \pm 1.2 ^A | 3.9 \pm 3.0 ^b | 1.9 \pm 0.3 ^B |
| Σ n-3 | 10.7 \pm 11.6 ^a | 9.1 \pm 2.3 ^A | 0.8 \pm 0.7 ^b | 1.9 \pm 0.5 ^B | 21.6 \pm 7.3 ^a | 7.6 \pm 1.3 ^A | 4.9 \pm 3.9 ^b | 2.0 \pm 0.3 ^B |
| Σ n-6 | 1.5 \pm 1.7 ^a | 0.7 \pm 0.2 ^A | 0.1 \pm 0.1 ^b | 0.1 \pm 0.0 ^B | 2.6 \pm 0.8 ^a | 0.5 \pm 0.1 ^A | 0.5 \pm 0.4 ^b | 0.1 \pm 0.0 ^B |
| n-3/n-6 | 7.1 ^a | 13.2 ^A | 8.0 ^b | 17.1 ^B | 8.3 ^a | 16.3 ^A | 9.9 ^b | 18.7 ^B |
| Σ SFA | 12.0 \pm 12.3 ^a | 4.7 \pm 1.2 ^A | 0.8 \pm 0.7 ^b | 1.1 \pm 0.3 ^B | 24.5 \pm 8.7 ^a | 4.0 \pm 0.7 ^A | 5.7 \pm 4.8 ^b | 1.1 \pm 0.2 ^B |
| Σ MUFA | 6.8 \pm 6.9 ^a | 1.3 \pm 0.4 ^A | 0.4 \pm 0.4 ^b | 0.2 \pm 0.1 ^B | 20.6 \pm 8.6 ^a | 1.0 \pm 0.2 ^A | 4.5 \pm 4.3 ^b | 0.2 \pm 0.1 ^B |
| Σ PUFA | 12.6 \pm 14.0 ^a | 9.8 \pm 2.5 ^A | 0.9 \pm 0.8 ^b | 2.0 \pm 0.5 ^B | 25.2 \pm 8.4 ^a | 8.1 \pm 1.4 ^A | 5.7 \pm 4.5 ^b | 2.1 \pm 0.3 ^B |

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

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⁻¹ 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 |
| Σ 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 |
| Σ 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 |
| Σ 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 |
| Σ 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 |
| Total FA | 31.7 \pm 33.1 | 15.9 \pm 4 | 2.1 \pm 1.8 | 3.3 \pm 0.8 | 70.7 \pm 24.5 | 13.2 \pm 2.3 | 15.9 \pm 13.7 | 3.5 \pm 0.5 |

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

Pablo Brosset: Data curation, Investigation, Resources, Visualization, Writing review

Christophe Lebigre: Data curation, Investigation, Resources, Visualization, Writing review

Philippe Soudant: Data curation, Investigation, Resources, Writing review

Marie Vagner: Project administration, Resources, Writing review

Laure Pecquerie: Project administration, Resources, Writing review

Fany Sardenne: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing original draft

Conflict of interest statement

Authors declare to have no conflict of interest.

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Marie Vagner: Project administration, Resources, Writing review

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