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

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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 (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/100 g 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/100 g). 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.

Highlights

► 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 that in white muscle ► DHA bounded to phospholipids is higher in anchovy than in sardine fillet

Keywords : dark muscle, neutral lipids, polar lipids, fatty acids, European sardine, anchovy, biomolecule

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1. Introduction

Omega-3 and -6 long-chain polyunsaturated fatty acids (n-3 and n-6 LC-PUFA), and especially eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (ARA; 20:4n-6) are among essential dietary biomolecules involved in physiological functions in humans, such as cardiac and brain functioning, hormone synthesis, and immune response (Calder, 2018; Swanson et al., 2012). Due to the insufficient *de novo* synthesis capacities of these molecules by humans (Burdge and Calder, 2005), the World Health Organisation recommends a dietary daily intake of 250−500 mg of EPA+DHA, including to reduce oxidative damage of the brain (Butt and Salem, 2016; FAO/WHO, 2010). Bioavailability is the degree of absorption and utilization of a nutrient contained in a food, varying depending the physiological state of the organism. It is affected by the chemical form and interactions with other food components. In the case of DHA, some recent research suggests that its chemical binding form may affect its bioavailability: a higher fraction of

dietary DHA would reach the brain when esterified to (lyso)phospholipids (*i.e.*, polar lipids, that constitute cell and organelle membranes) rather than to triglycerides (*i.e.*, neutral lipids, that constitute energetic reserve) (works reviewed by Ahmmed et al., 2020; Sugasini et al., 2019). Although not systematically observed in humans (e.g., Ulven et al., 2011), such a difference could change the nutritional interest of a food. However, literature about the distribution of DHA between phospholipids and triglycerides is scarce in small pelagic fish.

Small pelagic fish such as European sardine (*Sardina pilchardus*, Walbaum 1792) and European anchovy (*Engraulis encrasicolus*, Linnaeus 1758) are among the richest species in EPA and DHA (*ca.* 400−1700 mg/100g) (Gladyshev et al., 2018). They are the largest group of species landed in marine fisheries (*ca.* a quarter of global landings; FAO, 2020), and intended to supply these molecules to the human population through direct and indirect consumptions as oils and meals for animal rearing (Gladyshev et al., 2018; Tacon and Metian, 2013). In the same way as most teleosts, small pelagic fish have two types of muscles: the red (or dark) muscle used during slow (aerobic) swim, and the white (or light) muscle used in fast (anaerobic) swim. Red muscle has a better ability to beta-oxidise lipids for metabolic energy than the white muscle. It is also present in a higher proportion than the white muscle in mobile fish species (McLaughlin and Kramer, 1991; Teulier et al., 2019). It constitutes a fifth to a third of the total muscular mass in pelagic fish such as Clupeidae (including anchovies and sardines) and Scombridae (Greer-Walker et al., 1975; Teulier et al., 2019). In some Clupeidae (e.g., Peruvian anchovy), the red muscle is richer in total lipids and in DHA (in g/100g) than the white muscle (Albrecht-Ruiz and Salas-Maldonado, 2015). In spite of its relatively high nutritional quality, the red muscle is sometimes whitened during industrial processes (e.g. Zaghbib et al., 2017) or removed before human consumption because of its distinctive taste and its poorer conservation caused by its high myoglobin and haemoglobin contents (Videler, 2011). However, in fillets of European sardine and anchovy, little is known on the contribution of the red muscle to the overall EPA+DHA content and on the proportion of DHA esterified to phospholipids.

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

2. Material & Methods

2.1. Fish and tissues sampling

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

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

2.2. Moisture analysis

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

2.3. Lipid extraction and fatty acid analysis

Lipid extraction and FA analysis were performed as described in Mathieu-Resuge et al. (2020) with slight modifications detailed below. Total lipids were extracted from *ca.* 10 mg of dry tissue with 6 mL of $CHCl_3$:MeOH (2:1, v:v) into glass vials. The neutral and polar lipids were then separated by solid phase extraction (SPE) at low pressure on a silica gel micro-column with elution by 10 mL of $CHCl_3$:MeOH (98:2, v/v) and 20 mL of MeOH, respectively. After addition of an internal standard (23:0, in free FA form), both lipid fractions were dried in an EZ-2 evaporator (Genevac). After hydrolysis in 1 ml of KOH-MeOH (0.5 M) for 30 min at 80°C, samples were transesterified with 1.6 mL of MeOH:H_2SO_4 (3.4%; v/v) for 10 min at 100°C. FA methyl esters (FAME) were recovered in hexane and analysed on a TRACE 1300 gas chromatograph programmed in temperature and equipped with a splitless injector, a ZB-WAX column (30m×0.25mm IDx0.2µm) and a flame-ionisation detector (GC-FID, Thermo Scientific), using hydrogen as vector gas.

Obtained chromatograms were processed with Chromelon 7.2 (Thermo Scientific). Fifty-five FAME were identified by comparing their retention time with references from three commercial mixtures (37 components FAME, PUFA1 and PUFA3, Sigma), and in-house standard mixtures from marine bivalves, fish and microalgae GC-MS certified. Quantification of FAME was based on the internal standard recovery, and then FA contents were expressed in mass fraction of wet weight (mg g^{-1} ww and mg/100g ww) and in percentage (%) of total FA. Finally, the loss of fatty acids during the separation of neutral and polar lipids was verified by quantifying fatty acids on total lipids: the recovery rate was 91.6 ± 10.0% (n=9).

2.4. Data analysis

The differences in the FA composition (in %, considering only the FA representing >1% of total FA) were analysed by permutational analysis of variance (PERMANOVA, Anderson 2014) using Euclidean distances as dissimilarity values among individuals and considering species, muscle tissues and lipid fractions as factors. Principal component analyses (PCA) were performed to visualise the variation in FA profile composition between species (sardine and anchovy), muscle tissues (white and red muscles) and lipid fractions (neutral and polar lipids).

The FA content of fish fillet was calculated as follows, considering the relative proportions of red and white muscle:

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

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

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

3. Results

The FA profile (%) differed between the lipid fractions (PERMANOVA, df=1, r^2=0.82, p<0.001), between the species (df=1, r^2=0.04, p<0.01), between the lipid fractions of each species (df=1, r^2=0.02, p<0.01), and to a lesser extent between the muscle tissues (df=1, r^2=0.003, p<0.05). The two first axes of the principal component analysis explained 57% of the total inertia (39% on the first and 18% on the second component, respectively; Fig. 1). FA profiles of neutral and polar lipids were discriminated on the first principle component, while the two species were discriminated on the second principle component (Fig. 1). Polar lipids of both species were characterised by high proportions of LC-PUFA, such as ARA, n-6 DPA (docosapentaenoic acid, 22:5n-6) and DHA, while neutral lipids contained high proportions of monounsaturated FA (MUFA) and C18 PUFA.

Using Eq. 1 and results provided in Table 1, we found that the total FA content of sardine fillets was on average 2.9 times higher than that of anchovy (37.8 ± 15.1 *vs* 12.9 ± 7.9 mg g^{-1}, respectively; n=15 for each species, W = 9, p < 0.01; Fig. 2a). This difference was primarily due to neutral lipid FA content of fillets which was on average 3.3 times higher in sardine than in anchovy (25.4 ± 14.1 *vs* 7.7 ± 7.3 mg g^{-1}, respectively; W = 20, p < 0.01, Fig. 2a), while sardine fillets' polar lipid FA content was 0.9 times lower on average than that of anchovy (5.1 ± 0.7 *vs* 5.6 ± 1.2 mg g^{-1}, respectively; W = 157, p < 0.05, Fig. 2a). The total EPA+DHA content in fish fillet was on average 2.0 times higher in sardine than in anchovy (7.6 ± 6.7 *vs* 3.7 ± 5.1 mg g-1, respectively; W = 1372, p < 0.001; Fig. 2b). Again, this result was primarily due to neutral lipids EPA+DHA content which was on average 3.3 times higher in sardine than in anchovy fish fillets (10.5 ± 8.1 *vs* 3.2 ± 6.3 mg g-1, respectively; W =191, p < 0.01; Fig. 2b) while there were no differences in polar lipids EPA+DHA contents between species (4.5 ± 2.8 and 4.1 ± 3.5 mg g-1, for sardine and anchovy respectively; W = 580, p = 0.43; Fig. 2b). The total DHA content in fish fillet was on average 1.4 times significantly higher and more variable in sardine than in anchovy (5.8 ± 7.7 *vs* 4.1 ± 1.6 mg g^{-1}, respectively; W = 175, p < 0.01; Fig. 2c). The DHA content in neutral lipids of the fish fillet was on average 2.4 times higher in sardine than in anchovy, while in polar lipids it was 1.2 lower in sardine than in 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 *vs* 2.7 ± 0.6 mg g^{-1}, respectively; W = 41, p < 0.01; Fig. 2c).

In both species, the total FA content of the red muscle was always significantly higher than in the white muscle, whatever the lipid fraction considered (Table 1; Wilcoxon tests). Specifically, EPA+DHA content were higher in the red muscle than in the white muscle: EPA+DHA content in neutral lipids was significantly 13.6 and 4.4 times higher in the red

muscle compared to the white muscle for anchovy and sardine, respectively (Table 1; W = 332, $p < 0.01$ and W = 222, $p < 0.01$, respectively). The same difference was observed in polar lipids, with 4.7 and 3.7 times significantly higher EPA+DHA content in the red muscle than in the white muscle, for anchovy and sardine, respectively (Table 1; W = 450, $p < 0.01$ and W = 210, $p < 0.01$). Finally, DHA content in neutral lipids was 14.5 and 5 times higher in the red muscle than the white muscle for anchovy and sardine, respectively (Table 1; W = 336, $p < 0.01$ and W = 224, $p < 0.01$), and in polar lipids it was 4.8 and 3.5 higher (Table 1; W = 450, $p < 0.01$ and W = 201, $p < 0.01$).

4. Discussion

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

Fatty acid contents vary according to fish species and muscle types

In this study, sardine fillets contained about three times more of FA (or lipid) than those of anchovy. This may be explained by the higher proportion of the lipid-rich red muscle in sardine compared to anchovy. However, some environmental (e.g., season), and biotic (e.g., period of reproduction) factors can also influence the lipid content of both muscles between species. The influence of seasonality on the lipid content of fish from a given geographic area is commonly known and affected by the phenology of the species (Luzia et al., 2003; Pethybridge et al., 2014). It is also known that during the non-reproductive period (as it is the case for the studied individuals) clupeid species generally store lipids into muscles, which can then be mobilised toward gonads during the reproductive period (Brosset et al., 2015; McBride et al., 2015). These seasonal differences, largely documented for the white muscle (e.g. Pethybridge et al., 2014), may also occur in the red muscle. For instance, the red muscle of the Peruvian anchovy *E. ringens* is 2-fold richer in EPA+DHA during fall than during winter (Albrecht-Ruiz and Salas-Maldonado, 2015), probably linked to its reproductive cycle. Moreover, regardless of the season, the Peruvian anchovy contained a similar amount of EPA+DHA as the European anchovy in white muscle (*ca.* 0.8−1g/100g dw *vs* 1 g/100g dw

here) and red muscle (*ca.* 2.4−5.3 g/100g dw *vs* 4.6 g/100g dw here). It is thus necessary to consider the effect of seasonal changes on total lipid and EPA+DHA contents when examining the fish fillets as a source of LC-PUFA.

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

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

Distribution of fatty acids between lipid fractions

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

Conclusion

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

Ethical approval

Fish were sampled during the scientific surveys CGFS (September 2020) and EVHOE (October 2020), under European's data collection framework (DCF) and sampling

authorisation were provided by provided French national authorities (DPMA). Fish were dead by the time of sampling.

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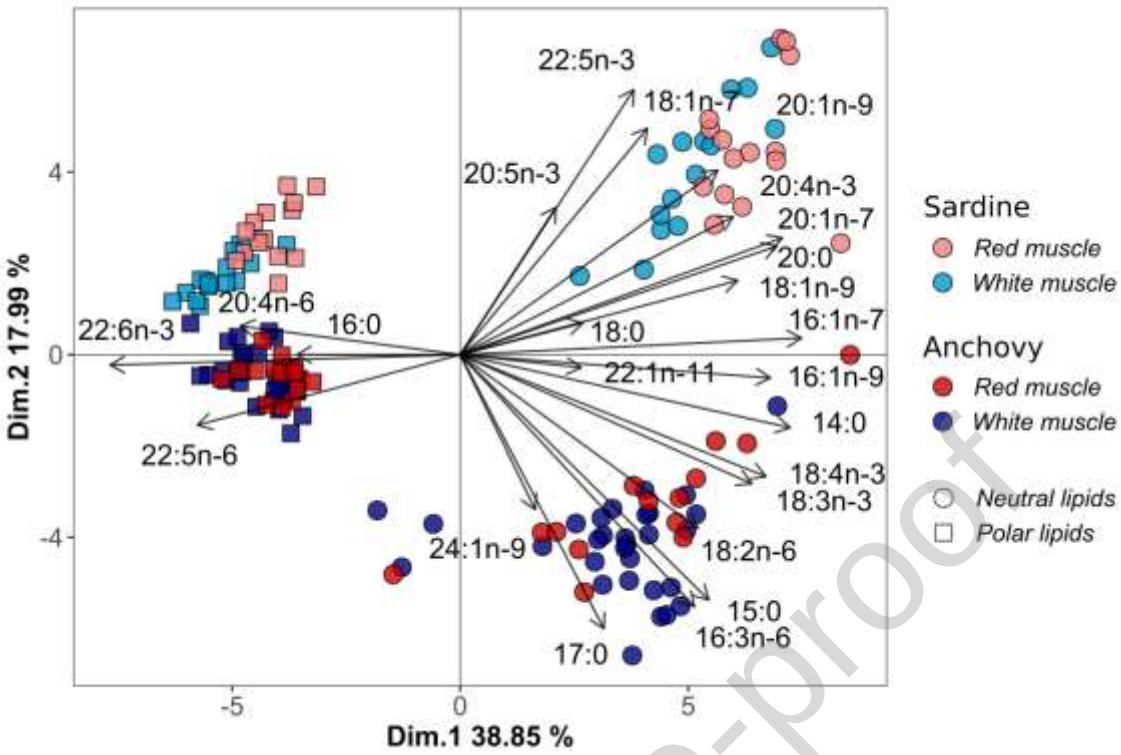

Figure 1. Principal component analysis (PCA) of the fatty acid composition (mass %) of neutral and polar lipids (displayed by dot shape), in red and white muscles (displayed by colours) of both species (sardine *S. pilchardus* and anchovy *E. encrasicolus*, displayed by colour intensity).

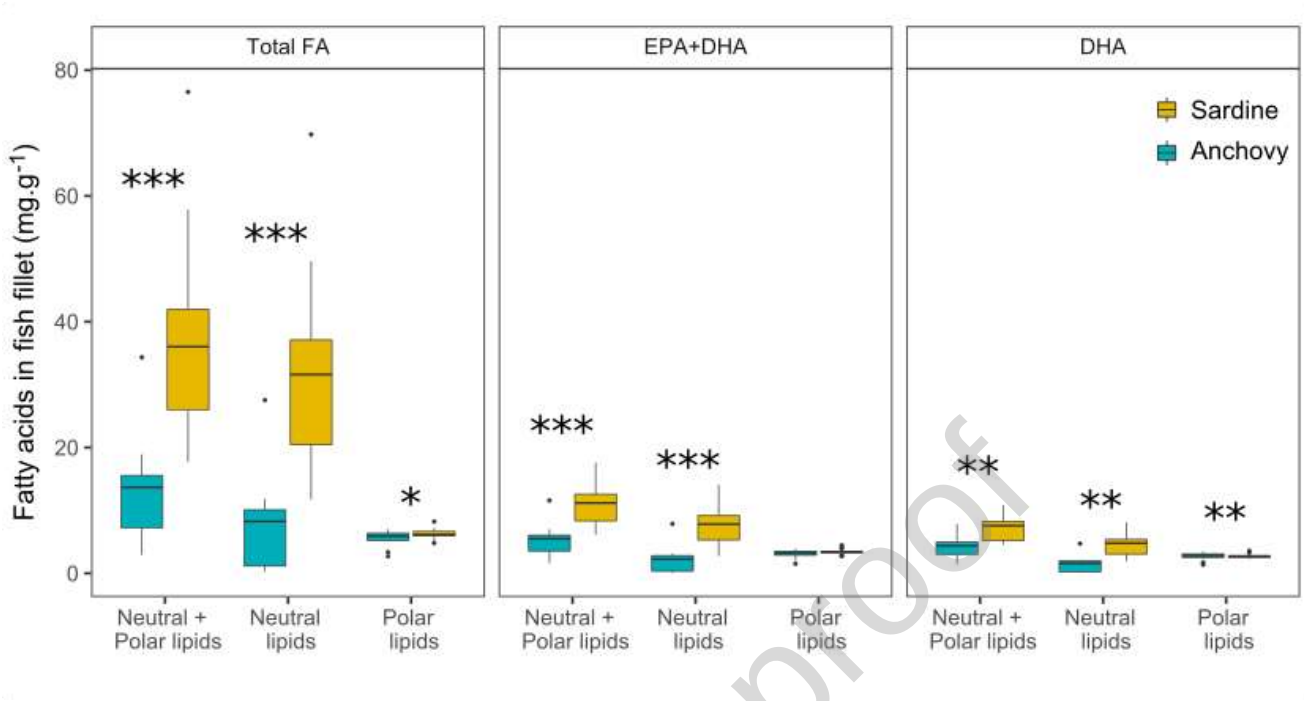

Figure 2. Total fatty acid, EPA+DHA and DHA contents (mg g^{-1}) in total, neutral and polar lipids of sardine *S. pilchardus* and anchovy *E. encrasicolus* fillets (*i.e.* considering the relative proportions of white and red muscles in the fillets). Significant inter-specific differences are indicated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

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

	Anchovy				Sardine			
	Red muscle		White muscle		Red muscle		White muscle	
Fatty acids	Neutral lipids	Polar lipids	Neutral lipids	Polar lipids	Neutral lipids	Polar lipids	Neutral lipids	Polar lipids
ARA	0.3 ± 0.4 ^{a}	0.2 ± 0.1 ^{A}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{B}	0.7 ± 0.2 ^{a}	0.2 ± 0.0 ^{A}	0.1 ± 0.1 ^{b}	0.1 ± 0.0 ^{B}
EPA	2.9 ± 3.6 ^{a}	1.0 ± 0.3 ^{A}	0.2 ± 0.2 ^{b}	0.3 ± 0.1 ^{B}	6.7 ± 2.5 ^{a}	1.6 ± 0.6 ^{A}	1.7 ± 1.5 ^{b}	0.3 ± 0.1 ^{B}

DHA	5.8 ± 5.7 [a]	7.6 ± 1.9 [A]	0.4 ± 0.4 [b]	1.6 ± 0.4 [B]	10.5 ± 3.6 [a]	5.6 ± 0.9 [A]	2.1 ± 1.6 [b]	1.6 ± 0.3 [B]
EPA+DHA	8.7 ± 9.2 [a]	8.7 ± 2.2 [A]	0.6 ± 0.5 [b]	1.8 ± 0.4 [B]	17.2 ± 5.6 [a]	7.3 ± 1.2 [A]	3.9 ± 3.0 [b]	1.9 ± 0.3 [B]
Σ n-3	10.7 ± 11.6 [a]	9.1 ± 2.3 [A]	0.8 ± 0.7 [b]	1.9 ± 0.5 [B]	21.6 ± 7.3 [a]	7.6 ± 1.3 [A]	4.9 ± 3.9 [b]	2.0 ± 0.3 [B]
Σ n-6	1.5 ± 1.7 [a]	0.7 ± 0.2 [A]	0.1 ± 0.1 [b]	0.1 ± 0.0 [B]	2.6 ± 0.8 [a]	0.5 ± 0.1 [A]	0.5 ± 0.4 [b]	0.1 ±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 ± 12.3 [a]	4.7 ± 1.2 [A]	0.8 ± 0.7 [b]	1.1 ± 0.3 [B]	24.5 ± 8.7 [a]	4.0 ± 0.7 [A]	5.7 ± 4.8 [b]	1.1 ± 0.2 [B]
Σ MUFA	6.8 ± 6.9 [a]	1.3 ± 0.4 [A]	0.4 ± 0.4 [b]	0.2 ± 0.1 [B]	20.6 ± 8.6 [a]	1.0 ± 0.2 [A]	4.5 ± 4.3 [b]	0.2 ± 0.1 [B]
Σ PUFA	12.6 ± 14.0 [a]	9.8 ± 2.5 [A]	0.9 ± 0.8 [b]	2.0 ± 0.5 [B]	25.2 ± 8.4 [a]	8.1 ± 1.4 [A]	5.7 ± 4.5 [b]	2.1 ± 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)*

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Conflict of interest statement

Authors declare to have no conflict of interest.

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 that in white muscle
- DHA bounded to phospholipids is higher in anchovy than in sardine fillet