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How membrane fatty acids influence sardine size across diverse marine environments

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ABSTRACT

Differences in diet quality and quantity may influence trophodynamic processes in small pelagic fish. Yet, we currently lack direct and comprehensive information on how large-scale areas differ in dietary resources and the degree to which these differences influence fish physiological performances (i.e., growth), ultimately influencing entire fish stocks. Fatty acid composition is one of the bioindicator that can provide insights on how dietary provisions of essential lipids influence the structure of the membrane fatty acids and subsequently fish growth among contrasted habitats. To address this issue, we conducted a large-scale sampling of European sardine (*Sardina pilchardus*) a species with major socio-economic importance that plays a key role as an energy pathway linking lower and upper trophic levels in pelagic ecosystems. We sampled individuals from the Gulf of Lions (Mediterranean Sea), the Bay of Biscay, and the English Channel (Atlantic Ocean) of age-0 to −3 + and found clear spatial differences in the quantity and quality of dietary lipids. Sardines from the Gulf of Lions fed on trophic food web based on dinoflagellates, with greater proportions of DHA (22:6n-3; docosahexaenoic acid) in reserve lipids. Sardines' reserve lipids had important proportions of zooplankton biomarkers in the English Channel (e.g., 20:1n-9 and 20:1n-11), and diatoms biomarkers such as 16C fatty acids and EPA (20:5n-3; eicosapentaenoic acid) in the Bay of Biscay. The relationship between sardines' membrane fatty acid composition and individuals' length changed progressively with individuals' age, a result consistent across areas, indicating ontogenetic abilities between largest and smallest individuals. Before maturity, largest sardines had higher DHA proportions, followed after maturity by higher proportions of ARA (20:4n-6; arachidonic acid), EPA and DPA (22:5n-3; docosapentaenoic acid). Finally, the study highlights the importance of considering the quality and diversity of dietary resources to better understand how individuals cope with their physiological needs. It is thus important to consider combined aspects (e.g., diet quality and diversity, influence of particular nutrients on length) to better understand the underlying mechanistic processes influencing fish physiology, likely cascading to different expression of their life history traits and affecting fisheries stocks.

1. Introduction

The flow of essential nutrients across trophic levels is a key process for the ecosystem functioning and stability. Among these nutrients, lipids have important biological roles in growth and reproduction of vertebrates (Vassallo-Agius et al., 2001; Fuiman and Ojanguren, 2011). More specifically, long-chain polyunsaturated fatty acids (LC-PUFA, ≥ C20), such as ARA (arachidonic acid, 20:4n-6), EPA (eicosapentaenoic acid, 20:5n-3), and DHA (docosahexaenoic acid, 22:6n-3) cannot be biosynthesized by marine fish, while their deficiency can impair several life history traits and other components of performances (Hou and Fuiman, 2021; Hulbert et al., 2014; Závorka et al., 2023). Once assimilated by animals, dietary fatty acids can either be incorporated in cell membrane composition as phospholipids, or be stored in reserve tissues mostly as triglycerides (Arts et al., 2001; Hulbert et al., 2014; Martin-Creuzburg et al., 2012). In contrast to fatty acids used as energy

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stores, those incorporated into cell membranes play a crucial role in the functioning of organisms by maintaining the integrity of cell membranes and metabolic activity (Hochachka and Somero, 2002; Hulbert et al., 2014). In pelagic fish, multiple experimental studies have shown that decreasing quantity and changing composition of dietary LC-PUFA caused changed in membrane fatty acid composition (Martin et al., 2013) later impairing individuals' growth (teleost fish, Tocher, 2010) and reproduction capacities (Common snook, *Centropomus undecimalis*, Yanes-Roca et al., 2009). Therefore, understanding how the variation of key dietary molecules influences fish health can help to understand the processes underpinning populations, communities, and ecosystems dynamics.

Small pelagic fish, such as the European sardine (*Sardina pilchardus*, Walbaum 1792) are important sources of vital dietary molecules in pelagic food webs, linking lower and upper trophic levels (Coll et al., 2006; Cury et al., 2000). They also present health benefits for human consumption by providing essential trace elements and LC-PUFA (Mathieu-Resuge et al., 2023), as such, play important roles in both economic and food security sectors. In the last decades, multiple studies have shown contrasted sardine population status among French coastal shelf areas. In the Bay of Biscay and the Gulf of Lions, the body condition, growth rate, and size-at-ages of sardines have decreased substantially (Boëns et al., 2021; Saraux et al., 2019; Véron et al., 2020). In addition to the high mortality of older individuals (Duhamel et al., 2016), these processes led to a fall in populations' biomass and hence exploitable stocks, especially in the Bay of Biscay (Véron et al., 2020). Boëns et al. (2021) recently shown that there is high selective mortality in sardines of the Bay of Biscay, with individuals growing fast in their first year having shorter lifespans than slower growing individuals. In the English Channel, the monitoring of the phenotypic characteristics of sardine started since the set-up of the PELTIC survey in 2012 (Doray and Boyra, 2021) and no signs of morphometric nor biomass decline over this relatively short time span has been observed (ICES, 2022; Menu et al., 2023). Sardines of the English Channel are generally larger than those of the Bay of Biscay, likely due to the greater primary and secondary productivity of the English Channel and/or a selection pressure in favor of faster-growing and reserve-building individuals (Gatti et al., 2018). Overfishing and pathogens are unlikely to play a key role in the changes of these phenotypic characteristics because these changes in growth were not related to stocks' harvest rate (Saraux et al., 2019; Boëns et al., 2021; Van Beveren et al., 2016), but bottom-up processes are largely expected to be an important driver of these declines (Biton-Porsmoguer et al., 2020; Brosset et al., 2016; Menu et al., 2023; Thoral et al., 2021). More specifically, the variability in growth across regions is consistent with dietary differences in these respective environments, with the effect of zooplankton dominating the effect of temperature (Menu et al., 2023). Changes in the taxonomic composition or phenotypic characteristics of the resources that fish feed on, such as observed in the Mediterranean Sea (Brosset et al., 2016) and the Bay of Biscay (Grandremy et al., 2023), may result in a fluctuation in lipid quality (i.e., in term of LC-PUFA contents, (Pethybridge et al., 2014, Hixson et al., 2015)). Therefore, the alteration in quantity (i.e., in terms of lipid contents) and more importantly in quality (i.e., in term of LC-PUFA composition) of food resources available to sardines among areas are likely part of the explanation to the changes in sardine life history traits (Biton-Porsmoguer et al., 2020; Grandremy et al., 2023; Menu et al., 2023). In the context of contrasting sardine population health status, investigating their fatty acids' compositions should help to elucidate dietary and physiologically differences among French coastal shelf areas.

The study of fatty acid profiles has to date largely been limited to single population (e.g., Bertrand et al., 2022) and/or focusing on one specific phenotypic variability (e.g. few age classes, few individuals, short time span of data collection), leading to a sparse representativeness at larger spatial scales. Other studies focused on community level and often relied on a limited sampling (e.g., Biton-Porsmoguer et al., 2020), while inter-individuals' variances in lipid and more specifically in fatty acid contents can be important (Herceg Romanić et al., 2021; Pethybridge et al., 2014). Moreover, these previous studies described the total fatty acid contents of fish (e.g., Pacetti et al., 2013, Brosset et al., 2015) while this does not allow the characterisation of the reserve *vs* membrane fatty acid composition. Hence total fatty acid contents may reflect the food source available (i.e., fatty acids contained in reserve lipids) or the fish physiology (i.e., fatty acids contained in membrane lipids) and teasing apart these aspects might be critical if we are to understand the processes driving changes in small pelagic fish characteristics. These markers are invaluable to examine whether trophic bottom-up processes are acting on the phenotype of targeted species. Therefore, a broader approach is clearly needed to enable direct comparisons of reserve and membrane fatty acids' compositions among sardine populations inhabiting contrasted environments. Thus, we conducted a large-scale sampling (with individuals from the Gulf of Lions, the Bay of Biscay and the English Channel) and included fish of different stages of life (from age-0 to $-3+$). We tested whether the total reserve lipid contents and its fatty acid composition, reflecting both quantity and quality of the sardines' diet, differed among the three contrasted areas. Then, how the membrane fatty acids' composition, which is a key parameter determining fish physiological processes will explain fish total length depending on the different environmental conditions and while ageing.

2. Material and methods

2.1. Study areas, fish and tissues sampling

Sardines were collected during scientific surveys in September and October 2020 in three areas (Brosset et al., 2023); the Bay of Biscay (n = 70; EVHOE survey https://doi.org/10.18142/8), the English Channel (n = 98; CGFS survey https://doi.org/10.18142/11), and in the Gulf of Lions (n = 109; PELMED survey https://doi.org/10.18142/19). In each area (Fig. 1), fish were sorted and total length, weight, age, sex, and sexual maturity stage were estimated when possible (Table 1). Dorsal fillets were removed on-board and stored at −80 °C. Once onshore, a piece of white muscle was placed into vials to be stored back at −80 °C. For each individual, two otoliths sagittae were extracted on board and mounted in leukit for age reading. Fish age were estimated under a binocular microscope and classified in 4 age categories: age-0, age-1, age-2 and age-3 +. The moisture content of white muscle samples (expressed in percentage of wet weight) was estimated by weighing before and after a 72-hours freeze-drying (Christ Alpha 1–2 LD plus lyophilizer).

2.2. Fatty acids analyses

After freeze-drying, muscle samples were homogenized with a manual potter and stored at −80 °C before lipid extraction. To this end, approximately 10 mg of the homogenized powder was placed in pre-combusted glass vials to which we added 6 ml of solvent mixture ($CHCl_3$:MeOH, 2:1, v:v). As described in Mathieu-Resuge et al. (2023), lipid extracts were flushed under nitrogen gas, sonicated, vortexed and rested 24 h to ensure complete lipid extraction. Briefly, an aliquot of total lipid extract was used to separate neutral lipids (i.e., reserve lipids) and polar lipids (i.e., membrane lipids) by solid phase extraction at low pressure. Subsequently, after adding C23:0 as internal standard (free fatty acid form), both lipid fractions were transesterified, first with KOH and then with H_2SO_4, to form fatty acid methyl ester (FAME) that were then recovered with hexane. FAME were analyzed on a TRACE 1300 gas chromatograph (Thermo Scientific) programmed in temperature and equipped with a splitless injector, a ZB-WAX column (30 m × 0.25 mm IDx0.2 μm) and a flame-ionisation detector, using hydrogen as vector gas. Obtained chromatograms were processed with Chromelon 7.2 (Thermo Scientific). Sixty-six FAME were identified by comparing their

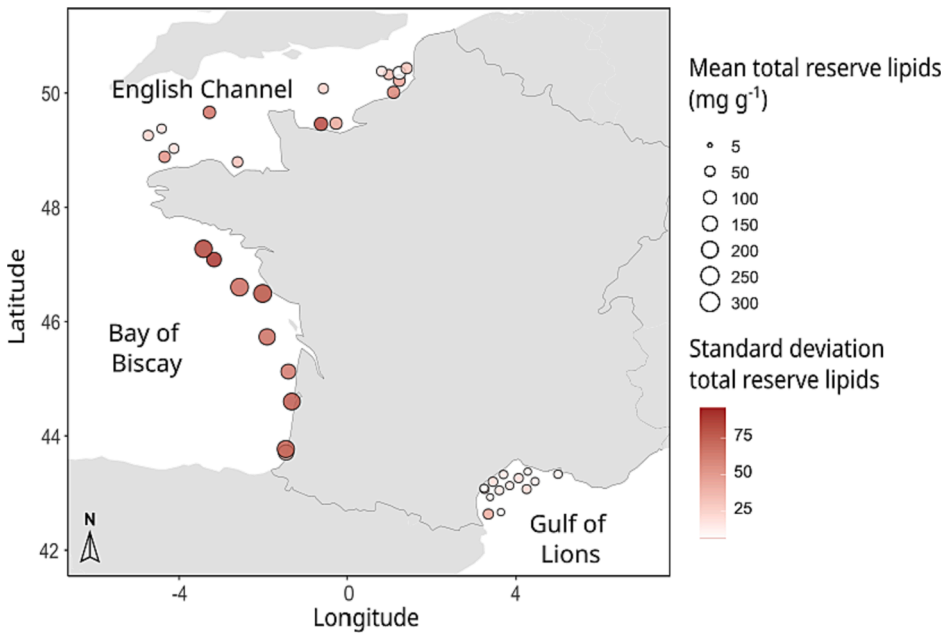

Fig. 1. Geographical distribution of the mean total reserve lipid content (mg g^{-1} dw) of individual sardines' white muscle per sampling station. The size of the dots represents the mean total reserve lipids and the color their standard deviation associated to each sampling station.

Table 1
Physical parameters (mean ± SD) measured on the European sardine (*Sardina pilchardus)* from the three studied facades.

	English Channel	Bay of Biscay	Gulf of Lions
Nb of fish	98	70	109
Age (y)	1.9 ± 0.8[a]	1.4 ± 0.9[b]	1.6 ± 0.9[b]
Weight (g)	73.5 ± 20[a]	40.2 ± 13[b]	13.1 ± 3.7[c]
Total length (mm)	204.7 ± 19.8[a]	170.9 ± 21.4[b]	121.7 ± 10.7[c]

retention time with references from commercial mixtures (37 components FAME, PUFA1 and PUFA3, Sigma) and from house-made standards. Quantification of FAME in µg was based on the internal standard recovery, and then expressed in mg g^{-1} of dry weight. Fatty acids relative proportions were expressed as mass percentages (%) of the total identified fatty acids.

2.3. Statistical analyses

We firstly examined the overall difference in reserve lipid contents among sampling areas (i.e., Gulf of Lions, Bay of Biscay, and English Channel) using a Kruskal–Wallis tests (KW, as normality distribution and homoscedasticity of data were not met) followed by Conover–Iman multiple comparisons applying a Bonferroni adjustment method (post-hoc tests). To compare overall reserve and membrane fatty acid profiles among areas, we used a permutational variance analyses (PERMANOVA). Comparison between each individual fatty acid contents (expressed in mg g^{-1} dw) among areas were ran using Kruskal–Wallis tests and post-hoc comparisons as previously described. We then performed principal component analyses (PCA) to quantify the overall variation in both reserve and membrane fatty acid compositions (expressed in %) between sampling areas. We used a similarity of percentages analysis (SIMPER) to identify the most discriminant fatty acids in reserve or membrane fatty acids; fatty acids accounting for more than 90 % of the dissimilarity were represented on PCA. We then extracted the two first principal components (PC1 and PC2) summarizing the overall fatty acid composition variability for subsequent analyses. Size difference of ellipses containing 95 % of individual, proxy of fatty acid composition diversity, were then compared using a betadisper analysis followed by an ANOVA. These analyses were performed separately for each PCA (individually ran on reserve and membrane fatty acids).

In a second step, we used linear models (LM) to test the relationship between reserve and membrane fatty acid composition (PCs) and the total length of sardines. These relationships have been tested for each age-group, sex, and sampling area. Total length has been chosen over size class to (1) not bias the study by the fact that all size categories are not equally represented in each area and (2) not decrease statistical power of analyses. Later, to determines whether relationships where consistent between sampling areas and ages, we included two-way interaction terms for sampling areas and age with PC1 and PC2. We selected the best model following a stepwise-backward selection and checked that Akaike's information criterion (AIC) clearly declined at each step (i.e., $\Delta AICc < 2$, Anderson and Burnham, 2004). All statistical analyses were performed with R v.4.2.2 (R Core Team, 2020). Fatty acid values are given as mean ± SE, and statistical tests were considered significant at $p < 0.05$.

3. Results

3.1. Sardines' reserve lipids and their fatty acid composition among areas

The sampled sardines differed in total length and weight, with heavier and larger individuals fished in the English Channel, followed by individuals from the Bay of Biscay and from the Gulf of Lions (Table 1). The total reserve lipid content (in mg g^{-1}) of sardines varied among sampling areas (KW test: $\chi^2 = 124.6$, $df = 2$, $p < 0.001$; Fig. 1 and Table 2). Reserve lipid contents in sardines from the Bay of Biscay were on average 3.3- and 6.4-times higher compared to sardines from the English Channel and the Gulf of Lions, respectively (Table 2).

The fatty acid composition (expressed in %) of sardines' reserve lipids varied significantly among the three areas (PERMANOVA: df = 2, F = 217.54, r2 = 0.61, $p < 0.001$) and these differences are highlighted in the PCA, separating the three areas (Bay of Biscay, Gulf of Lions, and English Channel, Fig. 2a). In this analysis, the first principal component (PC1) explained 23.4 % of the variance in fatty acid proportions and was positively related to 16:2n-4, 16:3n-4, 16:4n-1 and C18, C20 and C22 PUFA such as 18:2n-4,18:4n-3, 20:3n-6, 20:4n-3, EPA, and 22:5n-3 (DPA, docosapentaenoic acid) (Fig. 2a and contributions of the

Table 2
Reserve and membrane fatty acid composition (mean ± SD; mg g^{-1}) of the European sardine (*Sardina pilchardus)* from the three studied facades. Different letters indicate significant differences between facades among reserve and membrane lipids, respectively (significant level $p < 0.05$, tested by Kruskal Wallis, followed by followed by Conover–Iman multiple comparisons). Only the FAs accounting for > 1 % of total FA in at least one sample are shown, and mean values below 0.1 are reported as 0 ± 0.

	Reserve lipids			Membrane lipids		
	English Channel	Bay of Biscay	Gulf of Lions	English Channel	Bay of Biscay	Gulf of Lions
14:0	3 ± 2.2 ^{a}	16.2 ± 7.2^{b}	3.1 ± 3.2 ^{a}	0.1 ± 0 ^{a}	0.1 ± 0.1^{b}	0.1 ± 0^{c}
15:0	0.4 ± 0.3 ^{a}	1.5 ± 0.5^{b}	0.4 ± 0.4 ^{a}	0 ± 0	0 ± 0	0.1 ± 0
16:0	12.8 ± 8.4 ^{a}	41.9 ± 16.7^{b}	6.6 ± 5^{c}	3.6 ± 0.7 ^{a}	4.6 ± 1.1^{b}	4 ± 0.6^{c}
17:0	0.3 ± 0.2 ^{a}	1.3 ± 0.4^{b}	0.3 ± 0.2 ^{a}	0 ± 0	0.1 ± 0	0.1 ± 0
18:0	3.4 ± 2.3 ^{a}	9.9 ± 3.9^{b}	1.3 ± 0.9^{c}	0.7 ± 0.2 ^{a}	0.9 ± 0.3^{b}	0.7 ± 0.1 ^{a}
20:0	0.4 ± 0.3 ^{a}	2.1 ± 1.1^{b}	0.1 ± 0.1^{c}	0 ± 0 ^{a}	0.1 ± 0^{b}	0 ± 0^{c}
Σ SFA	**20.6 ± 13.7** ^{a}	**74 ± 29.1**^{b}	**12.1 ± 9.7**^{c}	**4.5 ± 0.8** ^{a}	**5.9 ± 1.4**^{b}	**5 ± 0.7**^{c}
16:1n-7	3.2 ± 2.7 ^{a}	15.6 ± 8^{b}	2.4 ± 2.1^{c}	0.1 ± 0 ^{a}	0.2 ± 0.1^{b}	0.1 ± 0^{c}
18:1n-7	1.8 ± 1.5 ^{a}	6.2 ± 4.8^{b}	0.7 ± 0.5^{c}	0.2 ± 0 ^{a}	0.3 ± 0.1^{b}	0.2 ± 0.1^{c}
18:1n-9	7 ± 5.9 ^{a}	17.4 ± 10.4^{b}	1.5 ± 1.1^{c}	0.3 ± 0.1 ^{a}	0.5 ± 0.1^{b}	0.3 ± 0.1^{c}
20:1n-9	1.4 ± 1 ^{a}	4.6 ± 3.8^{b}	0.5 ± 0.6^{c}	0 ± 0	0.1 ± 0	0 ± 0
22:1n-9	0.3 ± 0.2 ^{a}	1.5 ± 3.3^{b}	0.1 ± 0.1^{c}	0 ± 0	0 ± 0	0 ± 0
22:1n-11	0.9 ± 1.2 ^{a}	3.1 ± 4.5^{b}	0.6 ± 0.8 ^{a}	0 ± 0	0 ± 0	0 ± 0
24:1n-9	0.5 ± 0.3 ^{a}	1.5 ± 0.6^{b}	0.2 ± 0.2^{c}	0 ± 0	0.1 ± 0	0 ± 0
Σ MUFA	**16.4 ± 12** ^{a}	**53.5 ± 24.6**^{b}	**6.5 ± 5.3**^{c}	**0.8 ± 0.2** ^{a}	**1.3 ± 0.3**^{b}	**0.8 ± 0.1** ^{a}
16:2n-4	0.2 ± 0.2 ^{a}	1.3 ± 0.8^{b}	0.1 ± 0.1^{c}	0 ± 0	0 ± 0	0 ± 0
16:3n-3	0.1 ± 0.1 ^{a}	0.5 ± 0.2^{b}	0.1 ± 0.1^{c}	0 ± 0	0 ± 0	0 ± 0
16:3n-4	0.2 ± 0.3 ^{a}	1.1 ± 0.8^{b}	0.1 ± 0.1^{c}	0 ± 0	0 ± 0	0 ± 0
16:4n-1	0.3 ± 0.4 ^{a}	1.8 ± 1.4^{b}	0.1 ± 0.1^{c}	0 ± 0 ^{a}	0.1 ± 0^{b}	0 ± 0^{c}
18:2n-6 (LIN)	0.5 ± 0.3 ^{a}	2.7 ± 1.2^{b}	0.4 ± 0.3 ^{a}	0 ± 0 ^{a}	0.1 ± 0^{b}	0.1 ± 0^{b}
18:3n-3 (ALA)	0.6 ± 0.4 ^{a}	2.4 ± 1.2^{b}	0.2 ± 0.2^{c}	0 ± 0 ^{a}	0.1 ± 0^{b}	0 ± 0^{c}
18:4n-3 (SDA)	1.2 ± 0.8 ^{a}	5.9 ± 3^{b}	0.4 ± 0.3^{c}	0 ± 0 ^{a}	0.1 ± 0^{b}	0 ± 0^{c}
20:4n-3	0.5 ± 0.4 ^{a}	1.8 ± 0.8^{b}	0.2 ± 0.1^{c}	0 ± 0 ^{a}	0.1 ± 0^{b}	0 ± 0^{c}
20:4n-6 (ARA)	0.5 ± 0.4 ^{a}	1.4 ± 0.6^{b}	0.3 ± 0.2^{c}	0.2 ± 0.1 ^{a}	0.2 ± 0.1 ^{ab}	0.2 ± 0.1^{b}
20:5n-3 (EPA)	6.4 ± 4.8 ^{a}	25.3 ± 12.5^{b}	2.8 ± 2^{c}	1.2 ± 0.3 ^{a}	1.9 ± 0.6^{b}	0.9 ± 0.2^{c}
21:5n-3	0.2 ± 0.2 ^{a}	1 ± 0.5^{b}	0.1 ± 0.1^{c}	0 ± 0	0 ± 0	0 ± 0
22:5n-3 (DPA)	0.9 ± 0.7 ^{a}	2.9 ± 1.3^{b}	0.3 ± 0.2^{c}	0.1 ± 0 ^{a}	0.2 ± 0.1^{b}	0.1 ± 0^{c}
22:6n-3 (DHA)	8.5 ± 4.8 ^{a}	28 ± 9.8^{b}	5.2 ± 3.3^{c}	6.6 ± 1.4 ^{a}	8.6 ± 2.5^{b}	7.9 ± 0.9^{b}
Σ PUFA	**21.6 ± 14.1** ^{a}	**82.5 ± 33.4**^{b}	**11.1 ± 7.5**^{c}	**8.5 ± 1.6** ^{a}	**11.5 ± 3.2**^{b}	**9.7 ± 1.2**^{c}
Σ Branched	0.5 ± 0.4 ^{a}	1.8 ± 0.7^{b}	0.3 ± 0.3^{c}	0 ± 0	0 ± 0	0 ± 0
Total	**59.5 ± 40.1** ^{a}	**213.2 ± 86**^{b}	**30.1 ± 22.1**^{c}	**13.9 ± 2.4** ^{a}	**18.9 ± 4.9**^{b}	**15.6 ± 2**^{c}

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; Branched = iso15:0, anteiso15:0, iso16:0, iso17:0 and anteiso17:0.

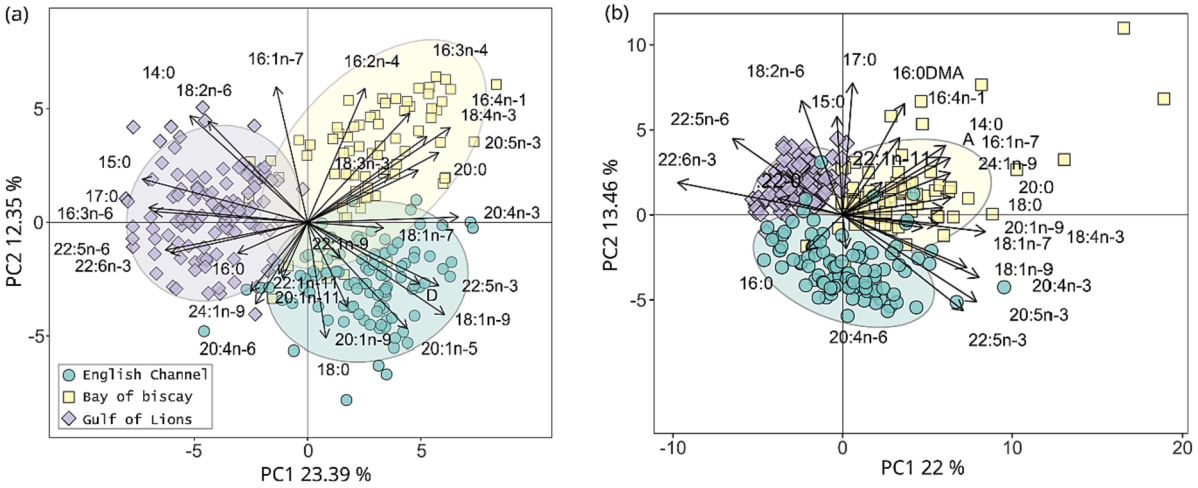

Fig. 2. Principal component analysis and associated ellipses discriminating the Gulf of Lions, Bay of Biscay, and English Channel, based on (a) neutral and (b) membrane fatty acid composition (expressed in %) of white muscle of sardine. Only fatty acids that account for >90 % of the dissimilarity between areas are shown (Simper test).

different reserve lipid fatty acids are described in Table 3). PC1 was also related to decreasing proportions of SFA (e.g., 14:0, 15:0 and 17:0), 16:3n-6, 22:5n-6, and DHA (Table 3). The second principal component (PC2) explained 12.4 % of the variance (Fig. 2a) and was related to increasing proportions of 17:0, 16:1n-7, 16:2n-4, 16:3n-4 and decreasing proportions of 18:0, 22:0, 18:1n-9, 20:1n-9, DPA, and DHA (Table 3). The spatial structure in fatty acid proportions was clear in this analysis as sardines sampled in the Bay of Biscay had generally positive PC1 and PC2 values, sardines sampled in the English Channel had generally positive PC1 values and negative PC2 values, and sardines sampled in the Gulf of Lions had negative PC1 values (Fig. 2a).

As marked by the difference in ellipses sizes, representing the

Table 3
Contributions of the different reserve and membrane lipid fatty acids to the two first PCA components. Only the most important fatty acids for each dimension (≥0.5) are shown and are the most important fatty acids for each dimension (≥0.6) are in bold.

	Reserve lipids		Membrane lipids	
	PC1	PC2	PC1	PC2
iso17:0	0.073	−0.131	0.578	0.214
14:0	**−0.598**	0.538	0.529	0.384
15:0	**−0.825**	0.204	−0.052	0.516
17:0	**−0.781**	0.042	0.031	**0.687**
18:0	0.173	**−0.606**	0.543	0.103
19:0	−0.372	0.124	−0.059	0.589
20:0	0.553	0.238	0.573	0.126
21:0	−0.342	**0,597**	0.001	−0.165
22:0	−0.186	**−0.556**	−0.351	0.028
24:0	**−0.598**	0.538	0.147	0.533
16:0DMA	**−0.580**	−0.152	0.279	0.594
16:1n-5	−0.456	**0.616**	−0.291	0.404
16:1n-7	−0.178	**0.702**	0.549	0.318
16:1n-9	0.505	−0.232	0.029	−0.044
16:2n-4	0.286	**0.685**	**0.817**	0.204
16:2n-6	−0.469	0.091	0.544	**0.656**
16:3n-4	0.501	**0.558**	**0.726**	0.229
16:3n-6	**−0.762**	0.032	−0.259	**0.692**
16:4n-1	**0.708**	0.490	0.254	0.390
18:1n-7DMA	NA	NA	0.305	**0.636**
18:1n-9	**0.685**	−0.467	**0.667**	−0.272
18:1n-9DMA	NA	NA	0.529	0.352
18:2n-4	**0.727**	0.260	**0.782**	−0.177
18:2n-6	−0.508	0.456	−0.225	**0.645**
18:3n-4	−0.047	0.416	−0.207	**0.627**
18:3n-6	0.128	0.279	0.577	0.373
18:4n-3	**0.617**	0.409	**0.747**	−0.101
20:1n-7	0.291	−0.426	**0.656**	0.191
20:2n-9	0.618	0.529	NA	NA
20:3n-6	**0.644**	−0.050	**0.696**	0.087
20:4n-3	**0.763**	−0.007	**0.720**	−0.324
20:5n-3 (EPA)	**0.667**	0.356	**0.708**	−0.451
21:5n-3	**0.846**	0.206	0.475	−0.327
22:1n-7	0.212	−0.364	**−0.605**	0.414
22:4n-6	−0.064	0.294	0.313	0.541
22:5n-3 (DPA)	**0.573**	−0.316	**0.637**	−0.510
22:5n-6	−0.701	−0.175	−0.574	0.399
22:6n-3 (DHA)	**−0.664**	−0.175	**−0.878**	0.134
Variance	15.71	8.48	12.40	7.68
% of variance	23.81	12.84	22.15	13.71
Cumulative % of variance	23.81	36.65	22.15	35.86

variation within each sampling area (Fig. 2a), there were significant differences in the dispersion of the fatty acid composition of reserve lipids among sampling areas (betadisper: df = 2, F = 5.35, p = 0.005; ANOVA: df = 2, F = 287.04, r2 = 0.67, p = 0.001). Sardines of the Bay of Biscay had a significantly greater heterogeneity of their reserve lipids' fatty acid composition compared to individuals from the English Channel (Tukey test: diff = 0.54, p = 0.027) and the Gulf of Lions (Tukey test: diff = -0.63, p = 0.006). However, individuals from the English Channel and the Gulf of Lions did not present significative differences in the dispersion of their fatty acid compositions of reserve lipids (Tukey test: diff = -0.09, p = 0.87).

3.2. Differences in membrane fatty acids among areas

Overall, sardines from the Bay of Biscay had 1.4- and 1.2-times higher contents of membrane fatty acids, compared to sardines from the English Channel and the Gulf of Lions, respectively (Table 2). The main LC-PUFA in membrane lipids of sardines were EPA and DHA. EPA contents were 1.6 and 2.1 times higher in sardines from the Bay of Biscay. No significant differences in DHA content were observed with sardines from the Gulf of Lions, while the sardines from the English Channel had the lowest DHA values observed in membrane lipids (Table 2).

The fatty acid composition of sardines' membrane lipids varied among sampling areas (PERMANOVA: $df = 2$, $F = 38.37$, $r^2 = 0.22$, $p < 0.001$). The differences that we observed in membrane lipids were broadly consistent with those of reserve lipids, where the sardines from the Bay of Biscay were mainly opposed to sardines from the Gulf of Lions on PC1, while sardines from the English Channel were discriminated from the two others areas with lower PC2 values (Fig. 2b). PC1, explaining 22.2 % of the variance, was primarily related to increasing proportions of 16:2n-4, 16:3n-4, 18:1n-9, 18:2n-4, 18:4n-3, 20:1n-7, 20:3n-6, 20:4n-3, EPA, and DPA (Table 3). PC1 was also related to decreasing proportions of 22:1n-7 and DHA (Table 3). PC2 explained 13.5 % of the variance and was related to increasing proportions of 17:0, 16:2n-6, 16:3n-6, 18:1n-7DMA, 18:2n-6, and 18:3n-4 (Table 3).

The length of sardines among different age was significantly related with both PC1 and PC2 of the PCA run with membrane fatty acids but not with reserve fatty acids (no significant relationship with the reserve fatty acids were found; Supplementary Table 1). These relationships strongly varied between sardine's age, an effect consistent across sampling areas as shown by the non-significance of the interactions between PC1, PC2 and area (Table 4; contributions of the different polar lipid fatty acids are described in the Table 3). The model explained 92.3 % of the variance in sardines' length that had a negative relationship with PC1 at age-0, and this slope became progressively positive as sardines aged (Fig. 3). Thus, the largest individuals at age-0 and smallest individuals at age-3 + had higher 22:1n-7 and DHA proportions, and conversely smallest age-0 and larger individuals at age-3 + had greater proportions of 16:2n-4, 16:3n-4, 18:1n-9, 18:2n-4, 18:4n-3, 20:1n-7, 20:3n-6, 20:4n-3, EPA, and DPA (Fig. 3a and d). The length of sardines was also positively related to PC2 at age 0 (Fig. 4), indicating that largest individuals at age-0 had higher 17:0, 16:2n-6, 16:3n-6, 18:1n-7DMA, 18:2n-6, 18:3n-4 proportions (Fig. 4a). The magnitude of this relationship decreased at age-1 but remained positive (Fig. 4b), and became negative in sardines of aged 2 and 3+ (Figs. 4c and d).

5. Discussion

Sardines inhabiting the Gulf of Lions, Bay of Biscay, and the English Channel had clearly different fat contents highlighting contrasted access to food quantity. The quality of their diet also clearly differed, as the variation in the composition of LC-PUFA in reserve lipids was substantial. Considering the bioindicators studied, membrane fatty acid composition was one of the main factors explaining sardines' length and the strength and direction of this relationship changed progressively with age, suggesting ontogenetic abilities between largest and smallest individuals of a same age. Finally, the relationship between fish length and membrane fatty acid composition displays similar patterns across areas, suggesting strong specific physiological acclimatation of fish independently of environmental conditions.

5.1. Sardines have different access to food resources depending on their habitat

This study demonstrates spatial variations in lipid reserve contents among sardine populations. Sardines from the Bay of Biscay had the greatest total reserve lipid contents, followed by sardines from the English Channel, and sardines from the Gulf of Lions. Reserve lipids are the principal source of energy mainly stored by organisms after dietary assimilation (Tocher, 2003). Therefore, sardines from the different studied areas clearly did not have access to the same quantity of food resources and/or some sardines started to use their lipidic reserves to invest to other tissues, as this could be observed during reproduction (Albo-Puigserver et al., 2020; Garrido et al., 2007; McBride et al., 2015). The spawning phenology of sardines in these areas differs, peaking in January (in the Gulf of Lions), April (in the Bay of Biscay), and June (in the English Channel), with a putative second spawning period reported in the Bay of Biscay and the English Channel in September-October

Table 4
Identification of the most parsimonious models quantifying the effect of the age, sex, area, and the three first axis of PCA performed on membrane lipid fatty acids on the sardines' total length.

Response variable	Main effects	Dropped variable	Retained variables	Parameter estimate (SE)	t-value	Deviance	F-value	*P*
Sardine total length	(PC1 + PC2)*Age+(PC1 + PC2)*Area + Sex	Sex		NA	NA	506	2.46	0.10
	(PC1 + PC2)*Age+(PC1 + PC2)*Area	PC2:Area		NA	NA	73	0.35	0.71
	(PC1 + PC2)*Age + PC1:Area + Area	PC1:Area		NA	NA	108	0.52	0.59
	PC1 + PC2 + Age + Area + PC1:Age + PC2:Age		**PC1**	−2.58	−2.142	NA	NA	**0.02**
			PC2	7.41	2.90	NA	NA	**0.004**
			Age	NA	NA	42,191	137	**<0.001**
			Area	NA	NA	79,882	389	**<0.001**
			PC1:Age	NA	NA	3246	10.6	**<0.001**
			PC2:Age	NA	NA	3941	12.8	**<0.001**

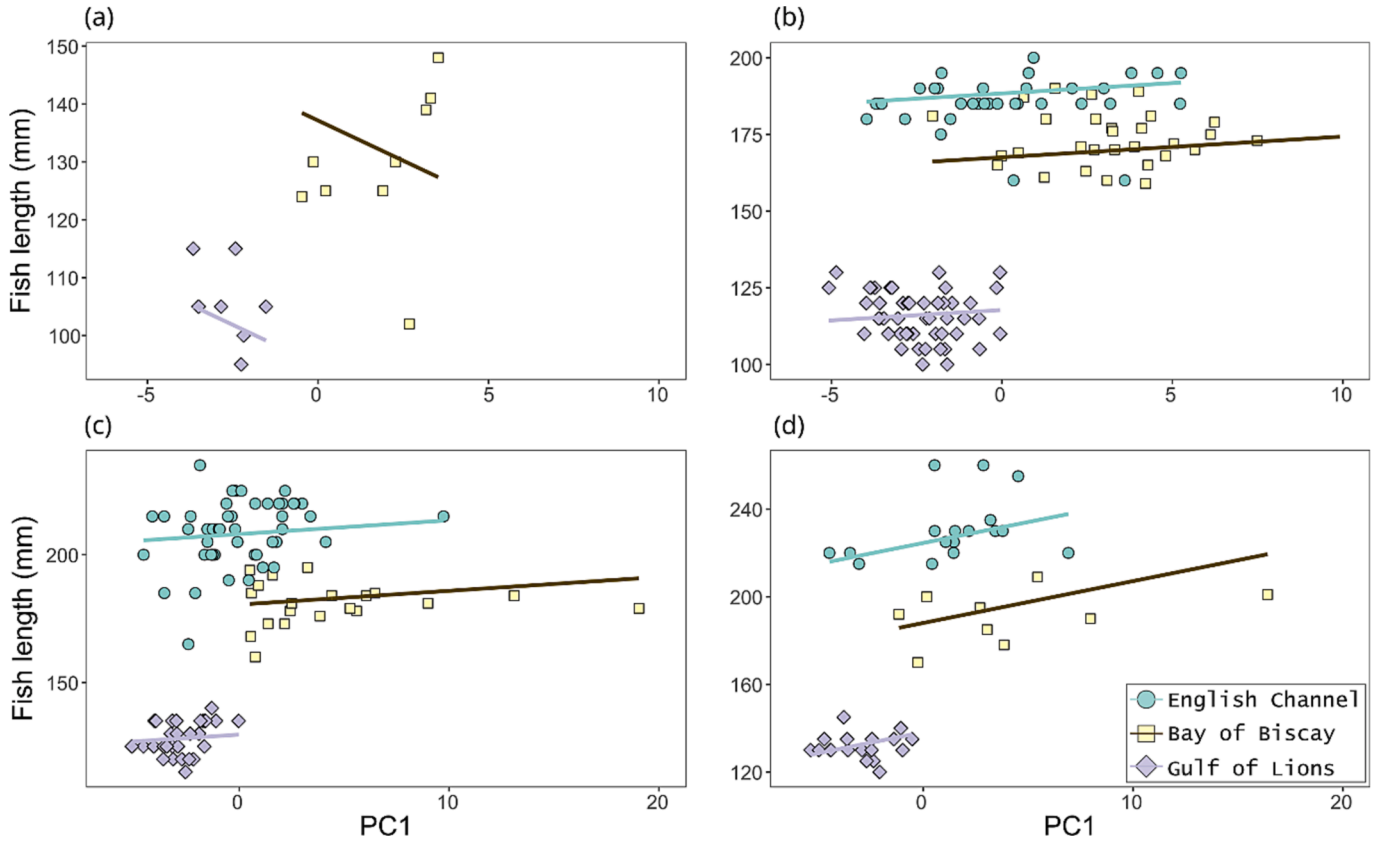

Fig. 3. Relationship between fish length (mm) and the first principal component (PC1) of PCA built using membrane fatty acids. Plots are separated by sardine ages: age 0 (a), age 1 (b), age 2 (c), and age 3+ (d).

(Menu et al., 2023; Saraux et al., 2019). However, none of the sampled fish was reproductively active at the time of sampling; sardines were sexually mature but displayed no gonadic development. As sardines produce multiple egg batches during the spawning season (i.e. indeterminate spawning), their vitellogenesis period is relatively short (Garrido et al., 2007) making it unlikely that the early onset of egg production in the Gulf of Lions had a substantial influence on our results. Therefore, the differences in the quantity of reserve lipids more likely reflect differences in the amount of food resources available in these different habitats, which can be themselves subject to seasonal changes (Albo-Puigserver et al., 2017). Lower lipid reserve contents can result to variety of physiological conditions of fish, likely cascading to a decline in the expression of life history traits (e.g., growth and reproduction). However, it has been suggested that the reduction in size of small pelagic fish is linked to a decline in food quality rather than quantity (Beauvieux et al., 2022; Queiros et al., 2019; Thoral et al., 2021). Therefore, there is a clear need to better describe differences in food quality among contrasting areas where fish do not dispose to the same access to food resources.

Sardines appeared to have access to variable proportions of essential nutrients (i.e., in term of LC-PUFA composition) depending on their habitat. Reserve lipids' fatty acid profiles reflect baseline food web and can reveal dominant food sources (Dalsgaard et al., 2003). We found that sardines fished in the Bay of Biscay had reserve lipids primarily composed of fatty acid reflecting trophic food webs based on phytoplankton communities composed of diatoms, as highlighted by the important proportions of EPA, and 16C fatty acids (i.e., 16:1n-7, 16:2n-4, 16:2n-7, 16:3n-4, 16:4n-1; Cañavate, 2019). Diatoms represent the highest abundance and biomass values of total phytoplankton in the Bay of Biscay (Muñiz et al., 2018). Previous studies have also shown that

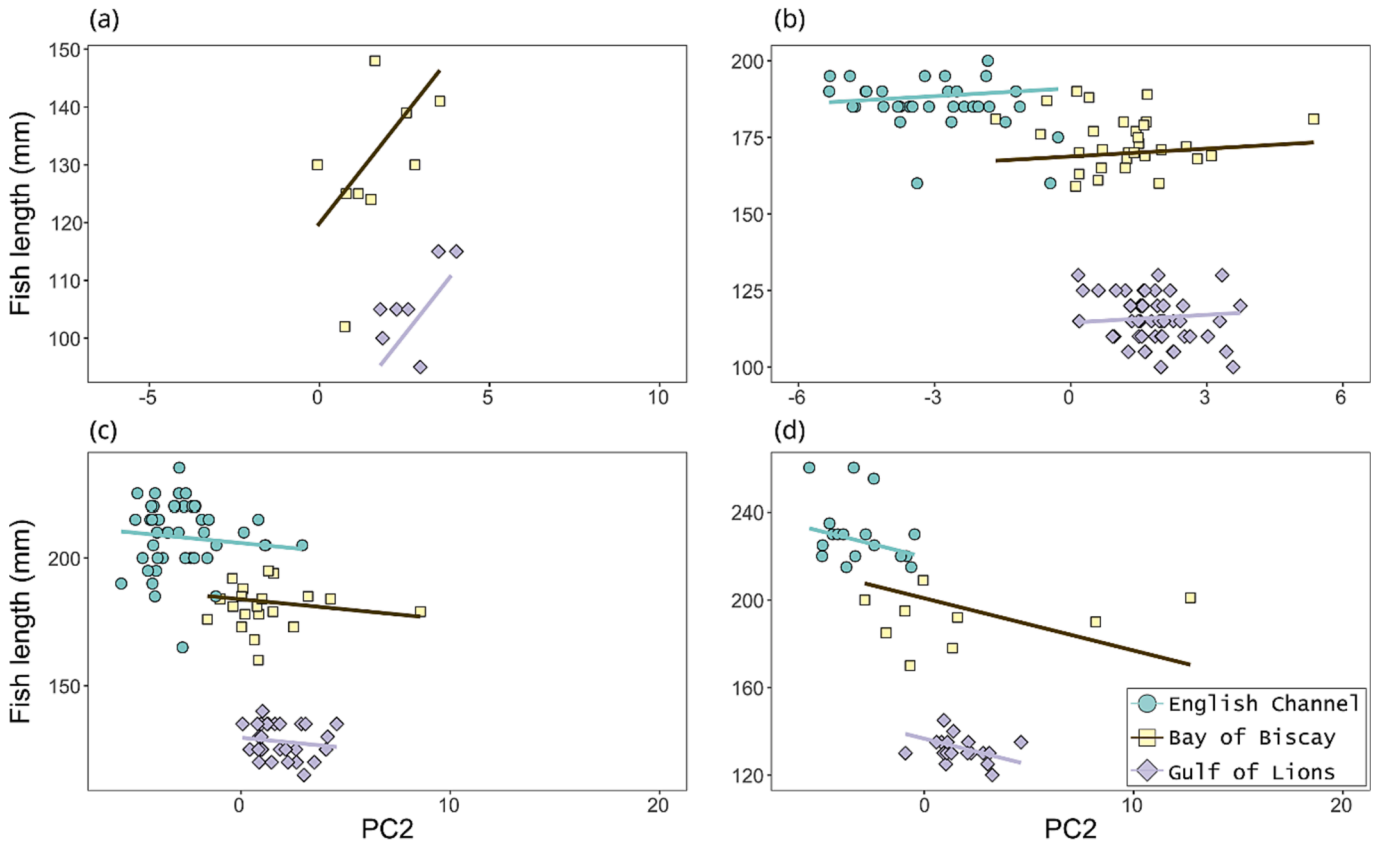

Fig. 4. Relationship between fish length (mm) and the second principal component (PC2) of PCA built using membrane fatty acids. Plots are separated by sardine age: age 0 (a), age 1 (b), age 2 (c), age 3+ (d).

diatoms can compose part of sardines' diet (Costalago et al., 2014; Le Bourg et al., 2015) and their predominance in sardines' diet coincides with observed autumnal blooms in this area (Lavender et al., 2008). Sardines of the Gulf of Lions fed on a trophic food web based on dinoflagellates, as shown by the higher proportions of DHA in their reserve lipids (Dalsgaard et al., 2003; Pethybridge et al., 2014). We also found that they had nine times lower EPA contents than those found in the Bay of Biscay. Previous studies have shown that the diet of Mediterranean sardines were based on diatom enriched in EPA in the early 2010's, some studies revealed that a switch to a diet predominantly based on dinoflagellates has occurred (Biton-Porsmoguer et al., 2020). The changes of primary producers in diet of sardines is likely due to environmental changes (Brosset et al., 2016; Menu et al., 2023), however changes of taxa is also cascading to different availability of essential nutrients to the entire food chains (Hixson et al., 2015; Hixson and Arts, 2016). EPA influences multiple physiological functions, and its dietary deficiency could be deleterious for organisms (Sargent et al., 1999). Sardines fished in the English Channel were feeding on food webs mainly composed of zooplankton and possibly copepods, as suggested by the high proportions of 20:1n-9 and 20:1n-11 in their reserve lipids (Falk-Petersen et al., 2002). In high latitudes, these fatty acids are often associated to copepod communities (Falk-Petersen et al., 2002, 2000), and to calanoids in the tropical Atlantic (Teuber et al., 2014), which are part of sardine feed (Bertrand et al., 2022; Costalago et al., 2014; Le Bourg et al., 2015). Marine zooplankton such as copepods present a high energy density (Brett et al., 2009; Falk-Petersen et al., 2002; Sargent and Falk-Petersen, 1988) and their deficiency can impact fish recruitment cascading to impairment of fish stock (Lomartire et al., 2021). It has been demonstrated that changes in prey size can have important bioenergetic consequences on sardines (Thoral et al., 2021), and our results demonstrate that sardines inhabiting different areas do not have access to the same quantity and quality of food. However, little is known on how change on taxa influence the transfer of essential nutrients and ultimately how their spatial distribution can subsequently explain differences of physiological functions, translating into differences in the expression of their life history traits among areas.

The sampling location was an important factor in determining the heterogeneity of fish food resources. Even though the fatty acid profiles of primary producers are phylogenetically defined (Jónasdóttir, 2019), their fatty acid contents may vary greatly by habitat and therefore affect omega-3 and omega-6 availability in food webs (Peltomaa et al., 2019). This study highlights that sardines from the Bay of Biscay had higher total neutral lipid content associated to a larger individual dispersion of their neutral fatty acid composition compared to sardines from the two others areas. The combination of a higher quantity and heterogeneity of food sources may suggest an access to a higher availability of food resources and/or larger sardine mobility. Such a high variability in fatty acids in sardines of the Bay of Biscay might at least partly be due to the strong and consistent spatial differences in zooplankton communities (Grandremy et al., 2023), suggesting that sardines in the Bay of Biscay may feed on very different preys depending on their location in the bay. Little is known on how the transfer of essential nutrients is affected by the taxonomic diversity of primary and secondary producers in aquatic ecosystem (Marzetz et al., 2017). However, the efficiency of these transfers can influence reproduction and offspring development of pelagic fish, both key for stock dynamic. Moreover, it is also important to consider dietary input variations at finer spatial and seasonal scales. Both can result in variations of food resources and quality (e.g., Bertrand et al., 2022), ultimately leading to changing individual body condition and life history traits energy allocation strategies (Beauvieux et al., 2022). Given the unprecedented rate of planktonic taxonomical diversity changes related to ecosystem alterations (Galloway and Winder,

2015; Hixson and Arts, 2016), it is essential to assess how the changes in diversity of food resources are influencing trophic transfers and the resulting supply of essential biomolecules to the entire food chain, including humans.

5.2. Membrane lipids' fatty acids are related to sardines' growth

Our results revealed the consistency of the relationship between essential membrane LC-PUFA and sardine length across sampling areas (i.e., none of the interaction terms between the PC1, PC2 and the sampling area were not significant). Thus, the biological processes underpinning membrane fatty acid composition and sardine's length relationships within each age are conserved over large spatial scales and populations, as the connectivity between the areas from North Atlantic and Gulf of Lions is limited (Caballero-Huertas et al., 2022). Membrane fatty acids such as the LC-PUFA ARA, EPA, and DHA are essential dietary requirements and limiting factors to physiological functions (Hulbert, 2008; Hulbert et al., 2014), including growth and reproduction (Sargent et al., 1993; Tocher, 2010; Závorka et al., 2023). The consistency of this relationship observed across areas indicates that membrane fatty acid composition might be a central process explaining differences in growth between individuals. Pinpointing the actual mechanism underlying this relationship is however complicated because multiple membrane fatty acid proportions are positively and negatively related to each PC (meaning that an increase in PC means greater values of some membrane fatty acids while lower values of some others). The sheer complexity of fatty acid composition resulting from differences in food availability, assimilation, and fatty acids selective incorporation among tissues must be considered jointly to fully explain this result. Therefore, experimental studies would now be required to better understand the biological significance of the membrane fatty acids and how they might change over individuals' lifespan.

Depending on sardines' age, the direction of the relationship between membrane fatty acid compositions and length changed, likely due to varying physiological needs over their lifespan (i.e., from growth to reproduction). Sardine muscles contain a large amount of membrane essential LC-PUFA such as EPA and DHA (Bertrand et al., 2022; Mathieu-Resuge et al., 2023). These essential components of cell membranes can be used and/or mobilized toward gonads during reproduction (Garrido et al., 2007), especially if the diet does not supply them in significant amount such as observed in the Gulf of Lions. However, at the time of sampling (September/October), sardines aged-0 are not yet mature (Silva et al., 2006) and mostly invest in growth and energy reserves accumulation before entering their first winter. Sardines with greater total length at age-0 had higher proportions of DHA but also lower 20:4n-3, EPA, and DPA levels in membrane lipids than smaller sardines (this result was particularly strong in sardines from the Gulf of Lion). DHA in larval fish is essential for the synthesis of physiologically important metabolites like prostaglandins, making it more important than EPA (Watanabe, 1993). However, an extreme deficiency in EPA does not allow to reach the optimal essential fatty acid ratios and might also be deleterious to fish larvae (Sargent et al., 1999). There is a clear selective mortality of sardines with rapid growth at age-0 in the Bay of Biscay (Boëns et al., 2021). Therefore, sardines that grew rapidly at age-0 may lack some essential membrane fatty acids such as the EPA, leading to poor essential fatty acids' tissue ratio, which might subsequently affect their ability to survive (Boëns et al., 2021; Menu et al., 2023).

This relationship declined at age-1, probably following the specific mobilization of DHA from muscles to gonads in pre-spawning period (early spring, gonads quickly develop to reach maturity in April-May), as it has been reported in the Japanese sardines (Yasuda et al., 2021). Indeed, there is significant variations in essential LC-PUFA contents (such as EPA and ARA) of Iberian sardine oocytes were induced by maternal effects, with a marked effect on egg quality and quantities of fat reserve available to larvae (Garrido et al., 2007). This specific accumulation is supported by our results and the fact that muscles of larger fish at ages 1 + accumulated more ARA and EPA than larger fish at age-0. These fatty acids are essential to gonads' development in small pelagic fish and are jointly accumulated in muscles and gonads during maturation (Garrido et al., 2007). Our results are also consistent with those of Garrido et al. (2007) who showed that EPA and DHA contents are negatively correlated in muscle. Larger fish of ages 1 + also accumulate more 20:4n-3 and DPA which are elongation products from the 18:4n-3 and EPA, respectively. These fatty acids are generally produced in low amount by marine plankton, therefore their important contents in sardine can suggest an effective elongation activity of fatty acids. This elongation activity can either reflect the deficiency in DHA (Bell et al., 1995), and/or be induced by the high proportions of biosynthetic precursors. However, if some studies revealed the presence of genes suggesting fatty acid biosynthetic capability of sardines, further studies are still necessary to fully describe the mechanistic processes involved (Emami-Khoyi et al., 2021; Machado et al., 2018; Sukumaran et al., 2023). Such a negative relationship between LC-PUFA could therefore point to a specific accumulation, re-allocation and/or biosynthesis of physiologically important LC-PUFA depending on the size of individuals. Thus highlighting concomitant changes in essential fatty acid proportions of individuals of a same age but differing in size to meet their physiological needs.

Membrane fatty acid composition and the relationship fish length varied within each age. This suggests that within each cohort, (i.e., all fish of the same age, in years), the physiological requirements of sardines for essential LC-PUFA vary according to fish size. The relationships between each PC and individual length within each age were broadly similar across sampling areas, suggesting that these effects probably reflect major differences in physiological needs rather than cohort effects (for which we could have expected the relationships to differ between sampling areas). Changes in morphometric parameters such as sardines' mean body length and weight have been monitored for a long time in all studied areas (Gatti et al., 2018; Menu et al., 2023; Saraux et al., 2019; Véron et al., 2020). Environmental variability in abiotic factors (e.g., Chlorophyll-a, sea surface temperature, salinity) alone could not explain the strong observed decrease in length and weight, while food quality may be a significant driver of the observed decrease in the Bay of Biscay and in the Gulf of Lions (Menu et al., 2023). Therefore, through bottom-up processes, the diet available to fish may explain differences in size observed within cohorts (Menu et al., 2023; Queiros et al., 2019; Thoral et al., 2021). The diet quality, expressed by the contents in physiologically relevant LC-PUFA are known to influence fish physiological performances (Závorka et al., 2023) and key life history traits such as growth (Vagner et al., 2015, 2014) from earliest life stages (Vagner et al., 2009). While these experimental approaches have not yet been transposed to sardines, the variation in dietary LC-PUFA availability found in our study likely plays a role in the difference in sardines' growth and selective mortality between in the three studied areas. Moreover, due to inter-individual variations in metabolism, differences in nutrients availability and the resulting disparity in the relationship between membrane fatty acids and fish size may also lead to different survival chances within a same cohort.

6. Conclusions

This study shows clear spatial differences in the quantity and quality of dietary LC-PUFA available to sardines, and that membrane LC-PUFA composition displayed similar age specific relationships with individuals' length across the three areas. Such a consistency across areas indicates common physiological needs to each population, independently of environmental differences. Moreover, these relationships differed at each age, highlighting that physiological requirement in LC-PUFA changed depending size and in association to reproductive stages. This study brings some possible explanations for sardine size selective mortality at large geographical scale by showing disparity in the proportions of essential LC-PUFA between largest and smallest fish from a

same cohort. However, this work needs to be pursued to fully understand which and how much dietary LC-PUFA are involved in physiological performances of sardines, and their specific influence on different life stages. Such effects are not only concerning sardine populations but may cascade to the entire food web, as European sardines play a key role in the transfer of energy between primary consumers and upper trophic levels. Consequently, a reduction in the quantity and quality of the energy content of small pelagic fish would not only impact the expression of their life history traits, but also threatens fishing and all the entire socio-economic activities that depend on these stocks.

CRediT authorship contribution statement

Margaux Mathieu-Resuge: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pablo Brosset:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Fany Sardenne:** Writing – review & editing, Visualization, Validation, Methodology, Data curation, Conceptualization. **Philippe Soudant:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Fabienne Le Grand:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Quentin Schull:** Writing – review & editing, Resources, Investigation, Conceptualization. **Christophe Lebigre:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

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Data availability

Data will be made available on request.

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Author contribution.

Conceptualization: MMR, PB, FS, PS, CL; Meetings/Discussion: MMR, PB, FS, FLG, PS CL; Methodology: MMR, PB, FS, FLG, CL; Validation: MMR, PB, FS, PS, FLG, CL; Formal analyses MMR, PB, CL; Investigation: MMR, PB, QS; Resources: QS, PS, FLG; Data curation: MMR, PB, FS, CL; Writing – original draft: MMR; Writing – Review and Editing: PB, FS, PS, FLG, QS, CL, Visualization: MMR, PB; Supervision: PS, CL; Funding acquisition: PB, CL.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pocean.2024.103209.

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