Spatial and ontogenetic variations in sardine feeding conditions in the Bay of Biscay through fatty acid composition

Mathilde Bertrand, Pablo Brosset, Philippe Soudant, Christophe Lebigre

Version of Record: https://www.sciencedirect.com/science/article/pii/S0141113621002701
Manuscript_a62486940c94363f78f40fd873f377a4

Spatial and ontogenetic variations in sardine feeding conditions in the Bay of Biscay through fatty acid composition

Mathilde Bertrand[1,2*], Pablo Brosset[1,2,3], Philippe Soudant[2], Christophe Lebigre[1*]

[1] Ifremer, Laboratoire de Biologie Halieutique, ZI Pointe du Diable - CS 10070, 29 280 Plouzané, France

[2] Université de Brest - UMR 6539 CNRS/UBO/IRD/Ifremer, Laboratoire des sciences de l'environnement marin - IUEM - Rue Dumont D'Urville - 29280 - Plouzané, France

[3] ESE, Ecologie et Santé des Ecosystèmes, Agrocampus Ouest, INRAE, 65 rue de Saint-Brieuc, 35042, Rennes Cedex, France

***Corresponding author:**

mathildebertrand@outlook.fr | Christophe.Lebigre@ifremer.fr

Ifremer, Laboratoire de Biologie Halieutique,

ZI Pointe du Diable - CS 10070, 29 280 Plouzané, France

Abstract

Food characteristics are amongst the most influential factors determining the fish life history traits as quantitative and qualitative changes in individuals' diet can lead to a decline in the energy allocated to their growth, and hence influence natural populations' characteristics. The size-at-age and weight of European sardines (*Sardina pilchardus*) in the Bay of Biscay (BoB) have decreased substantially over the last decade, especially for the youngest age classes, and the factors underlying such changes have not yet been identified. We therefore analysed the fatty acid (FA) composition in the neutral (NL) and polar (PL) lipids in samples collected across the BoB to determine whether the diet of sardines changes with their ages. We found that the

total FA contents in both lipid fractions varied mainly with the sampling locations and age. Indeed, sardines aged 1 and 2 years living in South BoB had particularly high contents in FA specific to non-diatom phytoplankton, while older sardines living in the Northern part had higher total FA content and more FA specific to copepods. These differences probably resulted from differences in prey availability and to a lesser extend a change in feeding behaviour with age. The strong dependence of younger sardines' diet to phytoplankton in spring suggests that changes in primary production may explain their decline in size-at-age. Finally, NL clearly reflect finest feeding variations in comparison to PL imprinted by diet variations at longer time scale. Future studies should consider separately NL and PL fractions.

Keywords: fatty acids, lipids, *Sardina pilchardus*, small pelagic fish, NE Atlantic, size-at-age

1. Introduction

Food characteristics are one of the most important biotic factors influencing individuals' fitness as it allows animals to extract energy from their environment. This energy is then allocated to life history traits and any alteration in its quantity or quality leads to a decrease in survival, growth, and reproductive success (Pigliucci, 2005; Stearns, 1992). Furthermore, the environment governs communities' composition and dynamic by controlling the abundance and assemblage of primary producers and their consumers (Dalsgaard et al., 2003; Hauss et al., 2012). For instance, Hixson and Arts (2016) and Pethybridge et al. (2015) predicted a decrease in the nutritional quality of phytoplankton along increasing sea surface temperature, potentially (i) affecting the recruitment and dynamics of forage fish (and their predators), and (ii) having consequences for human food safety (Budge et al., 2014) since fish may become less abundant and/or less nutritious. In other words, changes at the base of trophic chains can strongly influence the fitness of all species in the upper trophic levels, making it crucial to understand as such changes may have important consequences for stock productivity, ecosystem dynamics and fisheries yields (Carozza et al., 2019; Martino et al., 2019).

The European sardine (*Sardina pilchardus*) plays a central role in the transfer of energy between planktonic compartments and higher trophic levels in pelagic food webs (Certain et al., 2011; Cury et al., 2000). In the Bay of Biscay (BoB), there has been a marked decrease in size-at-age, weight-at-age and body condition of sardine (Doray et al., 2018b; Véron et al., 2020a, 2020b), a pattern similar in another small pelagic fish at the same trophic level, the European anchovy (*Engraulis encrasicolus,* Doray et al., 2018b). More specifically between 2000 and 2015, mean length of sardine decreased particularly at age 1 fish (*ca.* from 18.5 to

15 cm, i.e. 20%) and body condition declined by 15% on average across all ages (Doray et al., 2018b; Véron et al., 2020a). However, their size-at-maturity (*ca*. 14 cm) did not change and their age-at-maturity remains at 1 year in the BoB (Véron et al., 2020b). These declines have started while the stock had a relatively low exploitation rate and the increase in harvest was unrelated to the decline in fish growth and selective mortality (Boëns et al., 2021), making it unlikely that the recent decline in morphometry of sardines in this area can be attributed to fisheries-induced evolution. Therefore, such a decline in size-at-age and body condition is more likely to be driven by environmental changes in the BoB, though the actual mechanism remains elusive (Véron et al., 2020a, 2020b). Moreover, these phenotypic trends are similar to those observed in the Gulf of Lions since 2008, in which food quantity and quality have been identified as the main drivers leading to the decline of sardines size and body condition and the subsequent collapse of its fishery (Brosset et al., 2017, 2015b; Saraux et al., 2019). As sardine's stock of the BoB sustains major French and Spanish fisheries (totalling 32,299 tons landed in 2018; ICES, 2019), it is important to determine whether bottom-up processes also explain the decline in size-at-age and body condition in this species.

A bottom-up control of the phenotypic characteristics of sardines may result from changes in their diet: a decrease in the quantity of food available per individual due to density-dependent competition (the survival rate of juveniles increases in this stock; Doray et al., 2018b; Van Beveren et al., 2014), and/or a decrease in the quality of food (as it seems to be the case with sardines of the Gulf of Lions; Bachiller et al., 2020; Brosset et al., 2016; Saraux et al., 2019). Sardines primarily feed on small species of zooplankton (copepods, decapods, cirripedes, fish eggs and cladocerans) and phytoplankton (diatoms and dinoflagellates) but whose contribution to individuals' diet varies depending on fish length, season and region considered (Costalago et al., 2015; Garrido et al., 2008a; Van der Lingen et al., 2009). Indeed,

during sardine spawning periods (October to May, with peaks in November and April; Gatti et al., 2017), their broad distribution appears fragmented by the presence of cold bottom water (Bellier et al., 2007). Thus, in Spring the biomass of sardines is higher along the coast in the Southern BoB, near the Loire estuary, and in the waters South-West of Brittany (Doray et al., 2018a). In general, this population structure coincides with the distribution of sardine eggs (Bellier et al., 2007; Petitgas et al., 2006) and younger sardines are usually located in the Southern BoB (Silva et al., 2009). In the BoB, the abundance of primary producers and zooplankton is also strongly structured spatially as the amount of chlorophyll-a is particularly high near the coasts and estuaries (Adour, Gironde, Loire; Huret et al., 2013) and large copepods are particularly abundant near the shelf-break of the BoB (Dessier et al., 2018). Studies based on stable isotopes and stomach contents showed that sardines' diet changed as they aged (especially in spring and summer; Bachiller and Irigoien, 2015; Costalago et al., 2012; Le Bourg et al., 2015) and that there was no significant spatial pattern in fish diet within the BoB (Chouvelon et al., 2014). However, the characterisation of lipids and their variation may allow us to learn more about the sardines' diet and the constraints exerted by the modification of planktonic communities on their biology. Indeed, lipids are a source of energy (neutral lipids, hereafter NL) and underpin the properties of cell membranes (polar lipids, hereafter PL; Hulbert et al., 2014; Tocher, 2003). Lipids comprise saturated and unsaturated carbon chains called fatty acids (FA) that are particularly useful biomarkers of organisms' diet (Cartes, 2011; Dalsgaard et al., 2003; Meyer et al., 2019; Riquelme-Bugueño et al., 2020). Indeed, the FA synthesis chains differ between zoo- and phytoplankton meaning that the presence or absence of some FA can reflect changes in sardines' diet (Graeve and Greenacre, 2020). Thus, FA have been used as qualitative and semi-quantitative food web biomarkers and have proven to be a valuable method to define food web relationships, trophic positioning,

and the dietary behaviours of marine species (Turchini et al., 2009; Xu et al., 2020). FA can also inform us about the reproductive status of fish as NL are strongly solicited during reproduction (Gatti et al., 2017; Rosa et al., 2010) and largest sardines may have a longer laying period due to their larger quantities of NL (Nunes et al., 2011; Zwolinski et al., 2001). Furthermore, marine fish must find essential fatty acids (EFA; e.g. n-3 and n-6 polyunsaturated FA at 20 and 22 carbons) in their food as they have little or no ability to synthesise them *de novo* (Ahlgren et al., 2009; Hulbert et al., 2014; Sargent et al., 1999). It has been shown that EFA deficiencies can affect many vital functions such as growth, survival, stress resistance, and immune system (Benítez-Santana et al., 2007; Izquierdo, 1996; Koven et al., 1990). Therefore, EFA composition and quantity can enable us to test if fish nutritional needs are fully satisfied, both qualitatively and quantitatively (Sargent et al., 1997). Consequently, the variation in sardines' FA composition and concentration reflects their food characteristics (Bandarra et al., 1997) and their phenology (growth or reproduction; Pacetti et al., 2013), making these markers invaluable to examine whether bottom-up processes are acting on the phenotype of this species.

The aim of this article is therefore (i) to characterise the FA composition of sardine muscles in both NL and PL fractions, (ii) to determine whether it is associated with sardine endogenous characteristics (sex, age, weight, sexual maturity) and/or spatial distribution in the BoB, and (iii) to understand the variations in sardine feeding conditions according to the different FA useful to identify prey groups. Considering the spatial structure in food resources (i.e. primary production and zooplankton) and sardines' age across the BoB, we hypothesised that *S. pilchardus* FA composition changes according with the sampling location and individuals' age and that these changes are more visible in the NL than in the PL, due to a different FA turnover rate between these two lipid fractions.

2. Materials and methods

2.1. Acquisition of sardine samples: PELGAS 2018

The PELGAS survey, led by Ifremer, takes place in the BoB every May since 2000. Its main purpose is to estimate the biomass of small pelagic fish by acoustic detection, to inform the European Union on the status of the pelagic stocks in this area (Doray et al., 2018b). At each sampling location, called station, the fish are sorted by species and size (three size classes are defined in sardines: < 15 cm; 15-20 cm; > 20 cm). Muscle samples were collected in five sardines per size classes at 16 stations in May 2018 (PELGAS 2018, https://doi.org/10.17600/18000419). As only one or two different size classes were observed per station, our sampling is less homogeneous than we would have desired (Figure 1, Table 1). Muscle is an interesting tissue in clupeid species as it stores most of the lipid reserves (Brosset et al., 2015a; Lloret et al., 2013) at fairly constant levels year round except at the beginning of the spawning season (May is the end of sardine spawning, Garrido et al., 2008a), and is recommended for human nutrition (omega 3 dietary supply; Pacetti et al., 2013). For sardines, the biological parameters routinely collected were: body length, total fresh weight, age, sex and sexual maturity. Age is determined by microscopic reading of otoliths' growth rings (calcified parts of the inner ear of fish; ICES, 2011). Sex and sexual maturity stages are determined by macroscopic analyses of the gonads and assigned such as: (1) immature, (2) developing, (3) pre-spawning, (4) spawning, (5) partial post-spawning, and (6) post-spawning (Véron et al., 2020b). The average sardine size-at-age and their standard deviations were: 15.0 ± 1.0 cm for age 1, 17.8 ± 1.1 cm for age 2, 19.4 ± 0.6 cm for age 3, 20.7 ± 0.7 cm for age 4,

21.0 ± 1.1 cm for age 5, 21.7 ± 1.3 cm for age 6 and older (one individual for ages 7, 8 and 10). In addition, a piece of muscle was taken from each selected sardine and stored in the freezer at -80°C until lipid analysis in the laboratory. Overall, we collected 100 individual muscle samples during the 2018 survey.

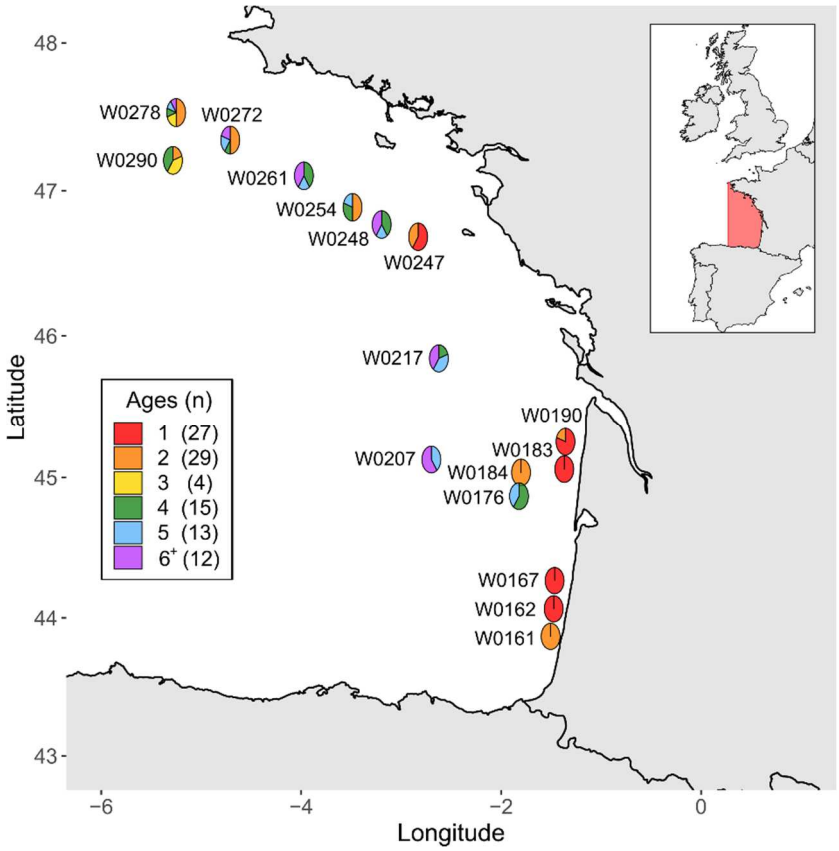


Figure 1. Spatial distribution of sardines sampled in the Bay of Biscay, as part of PELGAS 2018. Colours of the pie charts indicate the age of fish sampled for this study (with 6^+ individuals aged 6 years and over), and "n" is the number of individuals at each age.

Table 1. Geographical coordinates and biological parameters of sardines at the 16 sampling stations. S.D. is the standard deviation.

Station IDs	Latitude	Longitude	Number of individuals	Length (cm)		Weight (g)		Sex		Mean age (year)	Mean maturity stage
				Mean	S.D.	Mean	S.D.	Male	Female		
W0161	43.867	-1.505	5	17.0	0.0	37.8	4.6	5	0	2.0	5.0
W0162	44.065	-1.474	5	16.0	0.0	30.2	1.9	0	5	1.0	2.8
W0167	44.267	-1.465	10	15.0	0.5	25.6	2.1	7	3	1.0	3.1
W0176	44.868	-1.821	5	19.7	0.3	60.0	6.6	2	3	4.4	4.6
W0183	45.059	-1.368	5	14.0	0.6	20.6	3.6	2	3	1.0	2.6
W0184	45.036	-1.801	5	17.6	0.2	40.0	1.6	2	3	2.0	5.0
W0190	45.253	-1.356	5	14.0	0.0	19.8	2.0	4	1	1.2	2.2
W0207	45.127	-2.698	5	21.6	0.2	77.4	3.8	2	3	5.8	4.2
W0217	45.843	-2.623	5	20.3	0.3	62.6	3.4	2	3	5.2	5.0
W0247	46.685	-2.83	5	16.7	0.3	37.8	4.8	5	0	1.4	5.0
W0248	46.769	-3.195	5	22.0	1.4	82.8	12.5	1	4	5.8	5.0
W0254	46.887	-3.486	10	19.7	1.6	60.8	12.4	6	4	3.2	4.8
W0261	47.102	-3.973	5	21.0	0.4	76.4	10.9	2	3	5.4	5.0
W0272	47.345	-4.71	10	20.2	1.4	65.0	13.6	4	6	3.6	5.0
W0278	47.531	-5.251	10	19.9	2.3	67.1	23.9	6	4	3.1	5.0
W0290	47.207	-5.283	5	19.7	0.3	61.0	2.5	3	2	3.2	4.6

2.2. Grinding, lipid extraction and storage

Before any manipulation, the glassware was heated to 450°C for 6 hours and the metal or Teflon materials were rinsed with acetone to avoid contamination of the samples. We first solidified muscle samples in liquid nitrogen and passed them through a ball mill (1 min at 30 oscillations/sec). We retrieved between 200 and 250 mg of shred for each sample and added 6 mL of a $CHCl_3$/MeOH mixture (2/1, v/v) to extract lipids (Mathieu-Resuge et al., 2019). We then vigorously shook vials to re-suspend the shred and improve lipids' extraction. Prior to their storage at -20°C, the samples were passed under a flow of N_2, shaken, placed in an ultrasonic bath for 10 min, and agitated for at least 20 min.

2.3. Analyses of lipid classes

To ensure that samples were not degraded, we performed High-Performance Thin-Layer Chromatography (HPTLC) on total lipids (TL) based on Olsen and Henderson (1989). This TL plate allowed us to quickly visualise the different classes of PL and NL, including the free FA potentially appearing during the degradation of the samples. The samples analysed had no significant concentrations of free FA (<1% of TL) indicating that samples' degradation was absent or limited.

2.4. Fatty acid composition

To determine the composition of FA, lipid extracts stored at -20°C were first shaken for 20 min, centrifuged for 15 min at 3,000 rpm and 1 mL of each sample was transferred to new vials and evaporated to dryness. Dry lipid extracts were then re-suspended three times with 500 µL of $CHCl_3$/MeOH (98/2; v/v) and gently deposited on top of a silica micro-column (Marty et al., 1992). We eluted NL with 10 mL $CHCl_3$/MeOH (98/2; v/v) in a 22 mL vial and PL with 20 mL MeOH in another 22 mL vial (Le Grand et al., 2011). Upstream, an internal standard composed of 20 µL of C23:0 (0.115 µg/µL) has been added to each 22 mL vial. After the elution, the NL and PL fractions were evaporated to dryness using a Genevac centrifugal evaporator (program: Low Boiling Point; temperature: 30°C).

After evaporation, the dry NL and PL fractions were re-suspended three times with 500 µL $CHCl_3$/MeOH (2/1; v/v) and transferred into 7 mL vials. These were evaporated dry under N_2 flow and we then added 800 µL of H_2SO_4/MeOH (3.4%) and incubated the samples for 10 min at 100°C after vortexing (Budge et al., 2006; Le Grand et al., 2011). Heat and sulphuric acid catalyse the cleavage of ester bonds and methanol provided the CH_3 groups for the formation of FA methyl esters (FAME). We extracted FAME by adding 800 µL of hexane and

1.5 mL of hexane-saturated distilled water, and by shaking and centrifuging the NL and PL fractions 1 min at 1,000 rpm. FAME solubilise in hexane while the catalyst and glycerol mix with water, resulting in two phases. We discarded the denser aqueous phase and repeated this step twice by adding only 1.5 mL of hexane-saturated distilled water. Finally, the samples were delicately placed in the freezer at -20°C without removing the aqueous phase. After several hours, we quickly transferred the unfrozen upper organic phase into 2 mL vials, which are flushed with N_2 and stored in a refrigerator prior analysis.

We analysed our FAME one by one in gas chromatography coupled with a flame ionisation detector (GC-FID; Couturier et al., 2020) to quantify their concentration. Our samples were simultaneously analysed on two capillary columns (polar and apolar) to confirm the identity of FAME. The elution order of FAME is not the same on these two columns which produces two different chromatograms (one per capillary column).

The area of C23:0 (*ca*. 1,000 µV/min) has been checked using the software Galaxie Chromatography Data System (version 1.9.3.2) on all chromatograms to ensure proper reading of the samples by the GC-FID. Then, we assigned a FA to each peak by comparing the retention times of the two chromatograms of a sample (polar and apolar columns) with reference chromatograms (Couturier et al., 2020). The "NL FA" and "PL FA" data of the individuals were then processed by a predefined R script which calculates the mass (1) and mass percentage (2) of a given FA within NL and PL, based on the formulas below:

$$M_{FA,i} = \frac{A_{FA,i} \times M_{C23:0}}{A_{C23:0}} \quad (1) \qquad P_{FA,i} = \frac{100 \times M_{FA,i}}{\sum_{i=1}^{n} M_{FA,i}} \quad (2)$$

with M_{FA} the mass of a given FA, A_{FA} its area on the chromatogram, $A_{C23:0}$ the area of the reference FA (C23:0), $M_{C23:0}$ the mass of the reference FA (known as equal to 2.3 µg), and P_{FA}

228 the mass percentage of a given FA. The mass values obtained were finally related to
229 concentrations (µg FA/mg wet weight).
230
231 *2.5. Statistical analyses*
232
233 Statistical analyses were performed using R (version 3.5.1; R Core Team, 2018) and all
234 significance thresholds were set to $\alpha = 0.05$. First, we calculated the total amount of FA in both
235 NL and PL fractions to detect outliers and we characterised the FA of these two fractions by
236 quantifying inter-individual variations of the most important FA. We also calculated the
237 percentages of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and
238 polyunsaturated fatty acids (PUFA).
239 Due to the unbalanced age distribution in our sampling, we performed a canonical
240 redundancy analysis (RDA) to quantify the relationships between the composition of FA in NL
241 and PL and endogenous factors (i.e. sex, age, weight, sexual maturity) considering
242 geographical variables (i.e. stations' latitude and longitude) which constrained the FA data.
243 These geographical variables are interesting since they implicitly represent a gradient of
244 environmental conditions from the North to the South of the BoB and thus the effect of the
245 different endogenous variables was represented without effect of the environmental
246 variables. We then identified the overall structure of the data at the individual level and the
247 correlations between different FA with principal component analyses (PCA) performed
248 separately for NL and PL fatty acids, for which we extracted principal components (PC) with
249 eigenvalues >1 (this resulted in the extraction of 3 axes for each PCA). RDA and PCA were
250 based on 35 FA of our sardine muscles, where each FA was measured as µg/mg of wet weight
251 of sardine muscle. These were processed by the Hellinger distance (ideal for concentration

252 data; Legendre and De Cáceres, 2013) and were centred-reduced to give the same weight to
253 each FA. Among the 35 FA, we selected those >0.30% in TL (14:0, 15:0, 16:0, 17:0, 18:0, 16:1n-
254 7, 16:1n-9, 18:1n-7, 18:1n-9, 20:1n-9, 20:1n-11, 22:1n-9, 22:1n-11, 24:1n-9, 16:2n-4, 18:2n-6,
255 18:3n-3, 18:4n-3, 20:2n-6, 20:4n-3, 20:4n-6, 20:5n-3, 21:5n-3, 22:5n-3, 22:5n-6, 22:6n-3,
256 iso17:0) and some <0.30% in TL with ecological significance as biomarkers (20:0, 20:1n-7,
257 16:2n-7, 16:3n-4, 16:3n-6, 16:4n-1, 18:3n-4, iso15:0). Indeed, 16:2n-7, 16:3n-4, and 16:4n-1
258 belong to diatoms 16-carbon PUFA (Cañavate, 2019), 16:3n-6 is specific to some
259 *Chlorodendrophyceae* class (chlorophyta phylum; Jónasdóttir, 2019), iso15:0 belongs to
260 bacteria FA (Remize et al., 2020), and 20:0, 20:1n-7 and 18:3n-4 are potential indicators of
261 elongation activity (from 18:0, 18:1n-7 and 16:3n-4, respectively; Soudant, personal
262 communication).

263 To determine whether there was a spatial structure in sardines' diet at the scale of the
264 BoB, we performed dendrograms based on the dissimilarities in the values of the extracted
265 principal component axes between stations for each lipid fraction. These dissimilarities were
266 established based on Euclidean distances since our variables were quantitative and clusters
267 were identified using the "complete" method. To interpret RDA, PCA and dendrograms, we
268 focused on fatty acids trophic markers (FATM), which are specific to various groups of prey
269 and accumulate when consumed (Sargent, 1978; Sargent and Falk-Petersen, 1981).

270

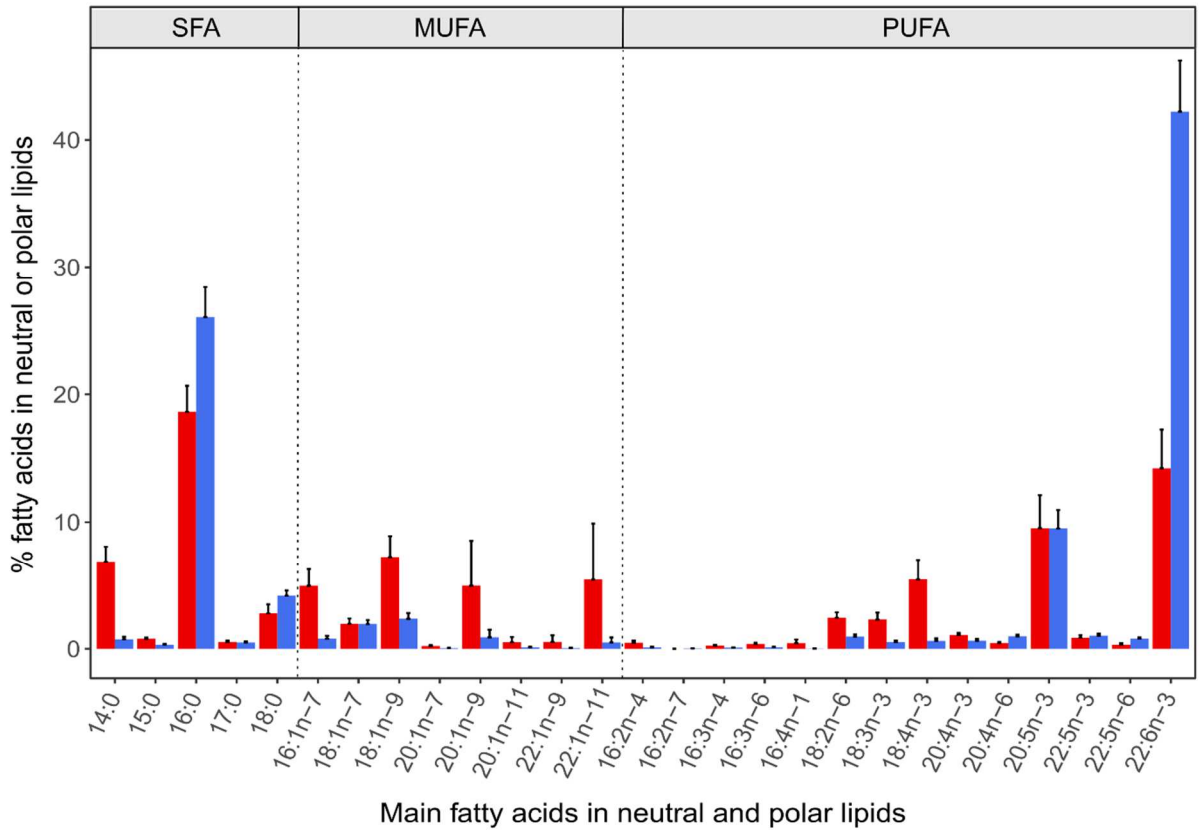
271 *2.6. Fatty acids trophic markers used*

272

273 In this study, the contribution of macrozooplankton to the diet of sardines was not
274 included due to the lack of specific enough FATM for this prey range (e.g. krill, decapods).
275 Therefore, we retained the FATM for three prey groups of sardines: copepods, diatoms, and

276 non-diatom phytoplankton. The FATM of herbivorous copepods such as *Calanus* spp. and
277 other calanoids (e.g. *Temora* spp.) are 20:1n-9 and 22:1n-11 (Kattner and Hagen, 1995); those
278 of diatoms are 20:5n-3 and 16-carbon FA such as 16:1n-7, 16:2n-4, 16:2n-7, 16:3n-4, 16:4n-1
279 (Cañavate, 2019); while others FATM such as 18:2n-6, 18:3n-3, 18:4n-3, 18:5n-3 and 22:6n-3
280 represent FATM from non-diatom primary producers (Dalsgaard et al., 2003; Napolitano et
281 al., 1997; Pethybridge et al., 2015). We also focussed on the EPA/DHA ratio (i.e.
282 eicosapentaenoic acid to docosahexaenoic acid, 20:5n-3/22:6n-3) commonly used as an
283 indicator of trophic relationships. This ratio decreases with increasing carnivory since DHA is
284 highly conserved in food webs (Dalsgaard et al., 2003; Scott et al., 2002).
285
286 **3. Results**
287
288 *3.1. Sardine fatty acids profiles in the Bay of Biscay*
289
290 Overall, we identified 56 and 57 FA in NL and PL fractions, respectively (4 FA were
291 specific to NL and 5 FA were specific to PL, Table S1). We found in NL 30% SFA, 27% MUFA,
292 and 40% PUFA (including 35% n-3 and 4.4% n-6, Table S1) whereas PL consisted in 32% SFA,
293 8% MUFA, and 59% PUFA (including 55% n-3 and 3.5% n-6, Table S1). The remaining
294 percentages are associated with dimethyl acetal FA (exclusively PL), branched FA, and
295 unknown FA (Table S1). The EPA/DHA ratio was 0.68 for NL and 0.23 for PL (Table S1). The FA
296 contributing most to the quantitative differences between NL and PL are: 14:0, 16:0, 16:1n-7,
297 18:1n-9, 20:1n-9, 22:1n-11, 18:4n-3 and 22:6n-3 (Figure 2). In general, there was a greater
298 inter-individual variability in NL than in PL for the 27 FA, since the average coefficients of

299 variation were 34% (ranging from 11% to 97%) and 29% (ranging from 9% to 80%), respectively
300 (Figure 2).
301



302

303 Figure 2. Percentages and standard deviations of 27 fatty acids in neutral lipids (red) and polar
304 lipids (blue) averaged for all individuals in the Bay of Biscay (n = 100). The fatty acids are
305 separated by category: SFA = saturated fatty acids, MUFA = monounsaturated fatty acids,
306 PUFA = polyunsaturated fatty acids.
307

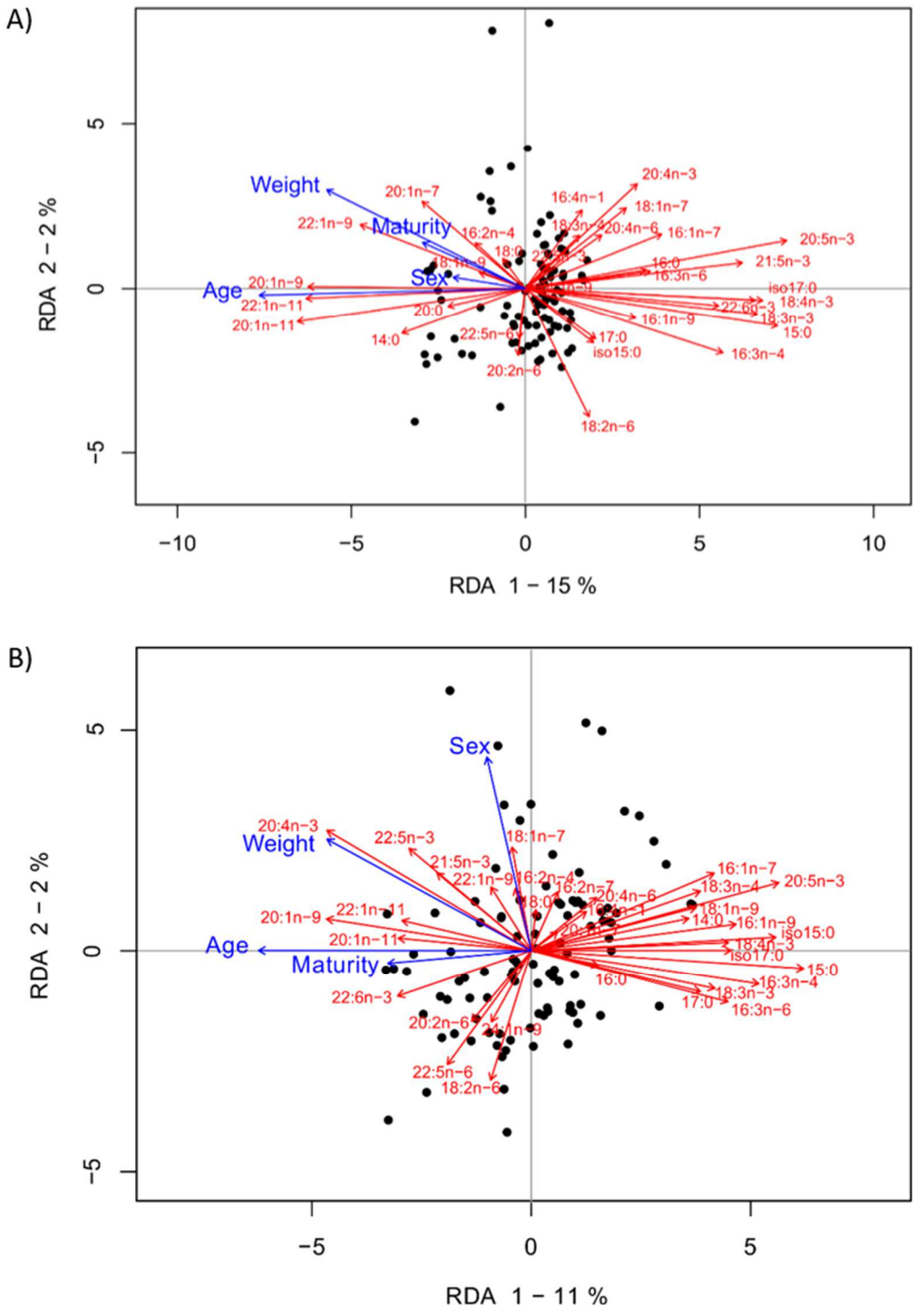
308 *3.2. The relationship between endogenous variables and fatty acids profiles*
309

310 The redundancy analyses highlighted that the contribution of the spatial and
311 endogenous variables was similar in NL (21 and 19% respectively, Table 2) while spatial

variables explained more variance than endogenous variables in PL (18 and 14% respectively, Table 2). While sardines' weight, sex and maturity stage explained a very limited amount of variance in FA, individuals' age explained nearly 20% of the total variation in FA (Table 2, Figure 3). More specifically, the increase in individuals' age is associated with major changes in the content of some FA (Figure 4 and Figure 5). In NL, we observed an increase in FA specific of copepods (20:1n-9 and 22:1n-11, Figure 3A and Figures 4B&C) and a decline in FA specific of phytoplankton (20:5n-3, i.e. diatoms, Figure 3A and Figure 4D). For other FA non-associated with specific prey groups, there were increases (e.g. 20:1n-11, Figure 4A) and declines with sardines' age (e.g. 15:0, 18:4n-3, Figures 4E&F). Some of these FA also changed similarly in PL (Figure 3B), which was particularly evident for the increase in 20:1n-9 (i.e. copepods, Figure 4H) and the decrease in 20:5n-3 (i.e. diatoms, Figure 4I) with sardines' age. As for NL, an increase (20:4n-3, Figure 4G) and a decline (15:0, iso15:0, Figure 4J&K) with sardines' age were observed for other FA non-associated with specific prey groups. We found a decrease in the EPA/DHA ratio with age in NL and PL fractions, although the ratio was three times greater in NL (Figure 5). Noteworthy, changes seemed to occur primarily between the sardines aged 1 and 2 and those older than 2 years old, both in NL and PL fractions (Figure 4 and Figure 5).

Table 2. Summary of the redundancy analyses quantifying the relationship between endogenous variables and fatty acid composition of sardines (i.e. constrained effect) after accounting for the contribution of geographic effects (i.e. conditioned effect). The percentage of variance explained by each effect and variable is reported in parentheses.

	Neutral lipids	Polar lipids
Conditioned	6.98 (20.5)	6.08 (17.9)
Constrained (λ1)	6.44 (18.9)	4.71 (13.9)
Unconstrained (λ2)	20.63 (60.6)	23.21 (68.3)
Variable	λ1/ λ2	λ1/ λ2
Age	0.26 (20.1)	0.16 (13.1)
Weight	0.01 (1.4)	0.01 (1.1)
Sex	0.03 (3.1)	0.03 (2.2)
Maturity stage	0.02 (2.3)	0.02 (1.9)



348

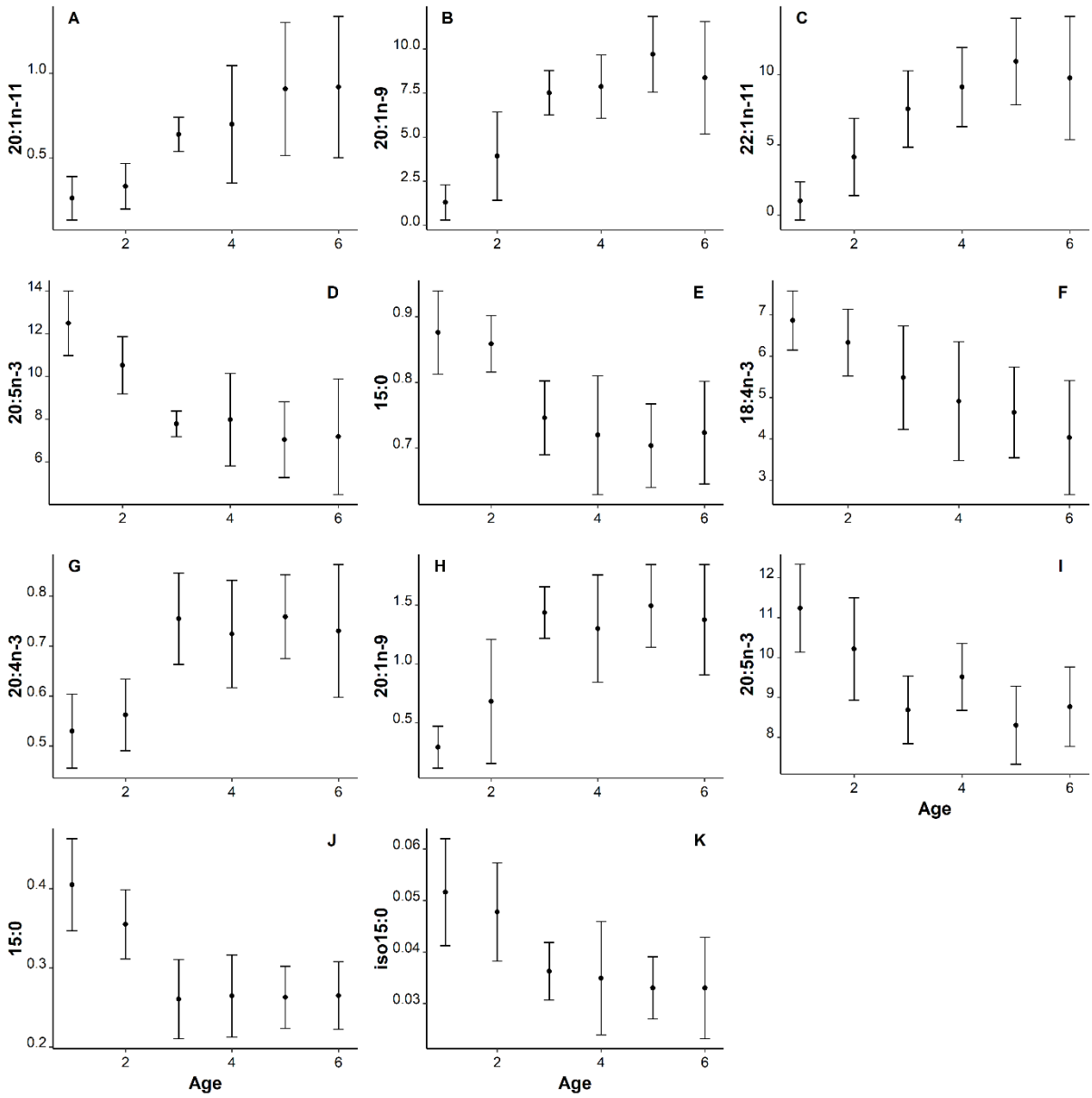
349 Figure 3. Redundancy analysis (RDA) ordination diagram for neutral lipids (panel A) and polar

350 lipids (panel B) based on the concentration of 35 fatty acids and the distribution of

351 endogenous variables, after accounting for the effect of sampling location.

352

353



354

355 Figure 4. Changes in the main age-related fatty acids identified by the redundancy analyses

356 (RDA) for neutral lipids (panels A to F) and for polar lipids (panels G to K). Age is provided in

357 years and fatty acids were measured as µg/mg of wet weight of sardine muscle.

358

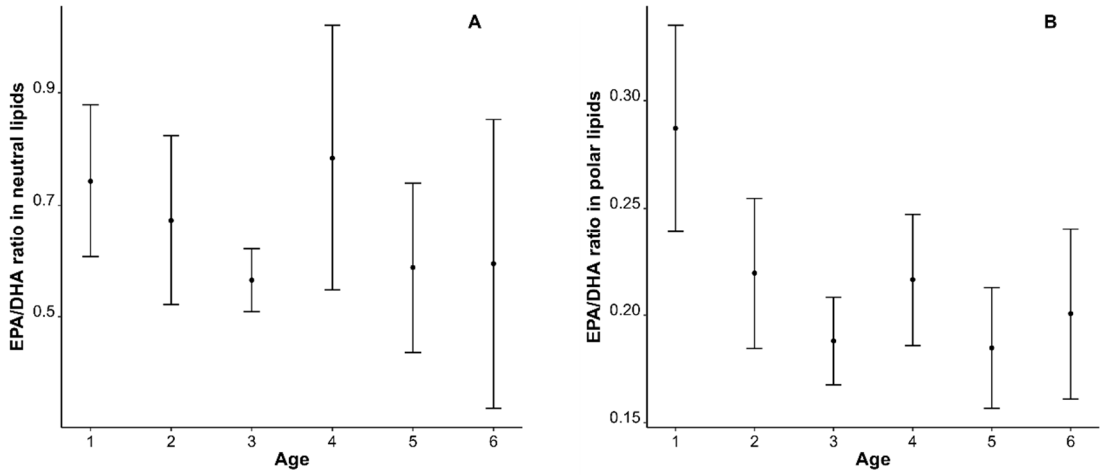


Figure 5. Changes in the EPA/DHA ratio according to the age of sardines for neutral lipids (panel A) and for polar lipids (panel B). Age is provided in years.

3.3. Spatial variability of fatty acids profiles

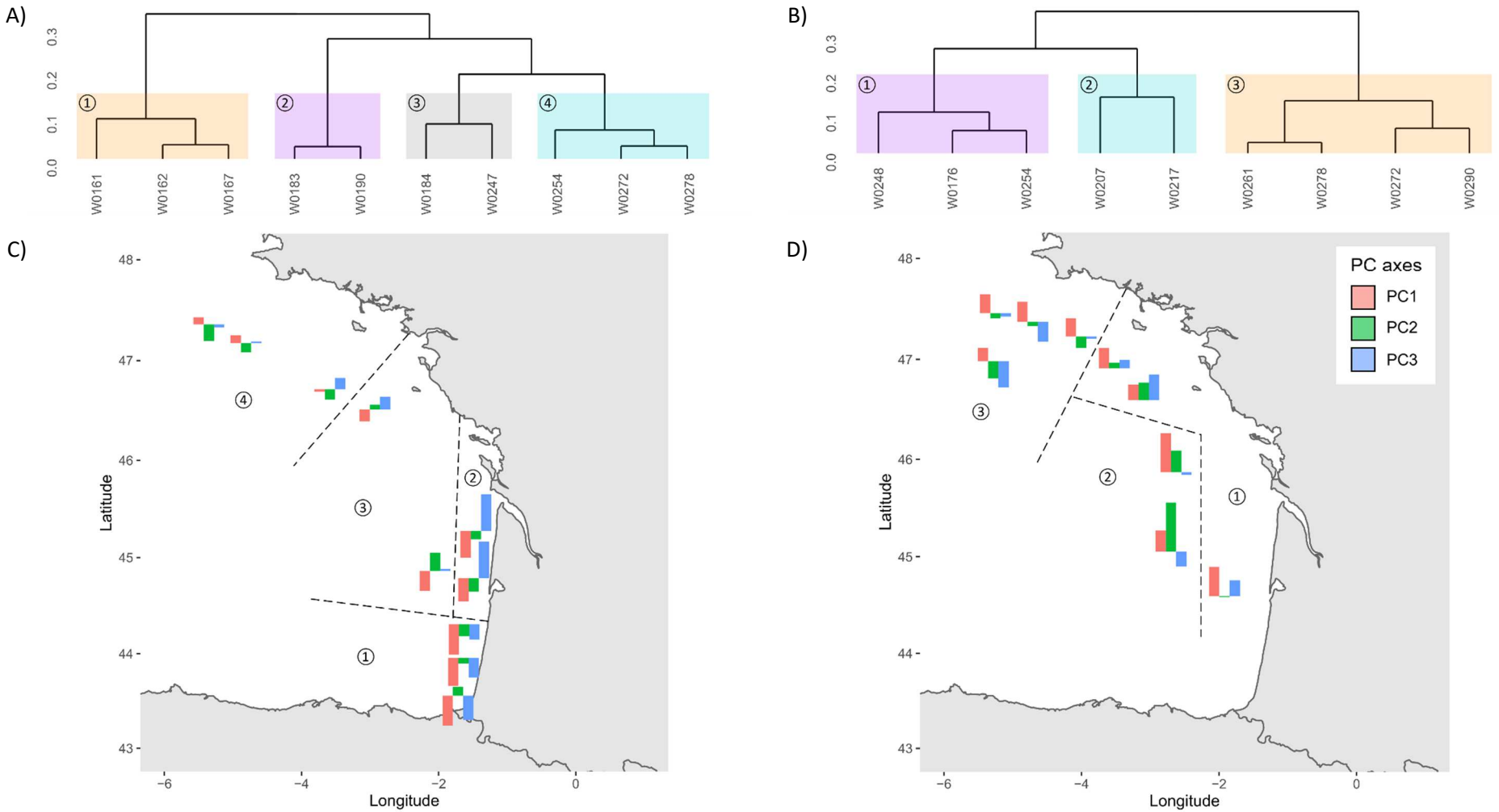
To determine whether there was a spatial structure in the 35 main FA, we first carried out PCA to produce integrative values of FA profiles for each individual. For NL, the first axis of the PCA (PC1_NL) was positively related to 20:1n-9 and 22:1n-11 (copepods FATM) and negatively related to 22:6n-3 and iso17:0 (non-diatom phytoplankton and bacterial FATM, respectively; Figure S1A and Table S2). The second axis (PC2_NL) was positively related to a mix of 20:5n-3, 18:3n-3 and 18:4n-3 (diatoms and non-diatom phytoplankton FATM) and negatively related to 22:5n-6 (non-diatom phytoplankton FATM mainly present in *Prymnesiophyceae*, *Pavlovophyceae, Pelagophyceae* and *Raphydophyceae*; Figure S1A and Table S2). The third axis (PC3_NL) was positively related to 16:4n-1, 16:1n-7 and 16:2n-4 (FATM typical of diatoms, Figure S1B and Table S2). For PL, the first axis of the PCA (PC1_PL) was negatively related to 16:1n-7, 16:3n-4 and 20:5n-3 (diatoms FATM) and positively related to 22:6n-3 (abundant in haptophytes and dinophytes, Figure S1C and Table S2), while the

second axis (PC2_PL) was negatively related to 20:1n-9 and 22:1n-11 (FATM of copepods) and 18:2n-6, 18:3n-3 and 18:4n-3 (non-diatom phytoplankton FATM, Figure S1C and Table S2). Finally, the third axis (PC3_PL) was negatively related to the diatoms FATM (i.e. 16:2n-7 and 16:4n-1) that were poorly explained by PC1_PL (Figure S1D, Table S2).

To account for the effect of ontogenetic diet changes in FA profiles, we carried out two separate clustering analyses (based on the values of the first three PCA axes) for NL and PL: one with only ages 1 and 2 sardines (10 stations) and another with older sardines (9 stations). For NL in 1 and 2 years old sardines, the clustering analysis identified four clusters of stations across the BoB (Figure 6A) distributed along a latitudinal gradient (Figure 6C). More specifically, the Southern group was characterised by primarily negative values of the PC1_NL (i.e. more non-diatom phytoplankton and bacterial FATM) and negative values of PC3_NL (i.e. less diatoms FATM; Figure 6C, area 1). The Northern group was characterised by positive values of PC1_NL indicating more FATM of copepods (Figure 6C, area 4). The third and fourth groups, located in between Southern and Northern groups, showed slightly negative and positive values for PC1_NL and PC3_NL, respectively, indicating both low concentrations of copepods FATM and more FATM of diatoms (Figure 6C, areas 2 and 3). The group located near the coast had particularly high PC3_NL (i.e. more diatoms 16-carbon FATM; Figure 6C, area 2) compared with the group further from the coast which was also characterised by positive values of PC2_NL (i.e. a mix of diatoms and non-diatom phytoplankton FATM; Figure 6C, area 3). We found a very similar spatial structure in sardines older than 2 years (Figures 6B&D) with the notable difference that PC1_NL values were all positive (depicting a large part of copepods FATM, Figure 6D). Stations located near the Gironde estuary were characterised by strong positive values of PC1_NL and PC2_NL (i.e. more copepods and a mix of diatoms and non-diatom phytoplankton FATM; Figure 6D, area 2). A second group included four stations in the

401 Northern part of the BoB in which sardines had also FA characteristic of copepods but low
402 values of PC2_NL and PC3_NL (i.e. more non-diatom phytoplankton FATM; Figure 6D, area 3).
403 A third group was identified near the coast and characterised by a more equal content of
404 FATM of copepods, diatoms and non-diatom phytoplankton (Figure 6D, area 1).

405 For PL, the hierarchical dendrogram for 1 and 2 years old sardines based on PCA axes
406 values distinguished two clusters (Figures 7A&C). Southern stations had negative and positive
407 values of PC1_PL and PC2_PL, respectively, indicating FA characteristic of diatoms (Figure 7C,
408 area 1). Northern stations had higher PC1_PL and lower PC2_PL indicating higher proportions
409 of non-diatom phytoplankton (PC1_PL and PC2_PL) and copepods FATM (PC2_PL; Figure 7C,
410 area 2). For sardines older than 2 years, we did not find any clear spatial structure in PL (Figures
411 7B&D).



412

413 Figure 6. Hierarchical dendrogram showing the clustering of stations (panels A&B) according to the fatty acid profile on the first three principal

414 components (PC) of PCA on the neutral lipids and their location in the Bay of Biscay (panels C&D) for ages 1 and 2 sardines (panels A&C) and age

3 and older sardines (panels B&D). The numbers indicate the different zones. The PC axes are defined from FATM as: PC1 positive: copepods, PC1 negative: non-diatom phytoplankton and bacteria, PC2 positive: diatoms and non-diatom phytoplankton, PC2 negative: non-diatom phytoplankton, PC3 positive: diatoms, PC3 negative: not applicable.

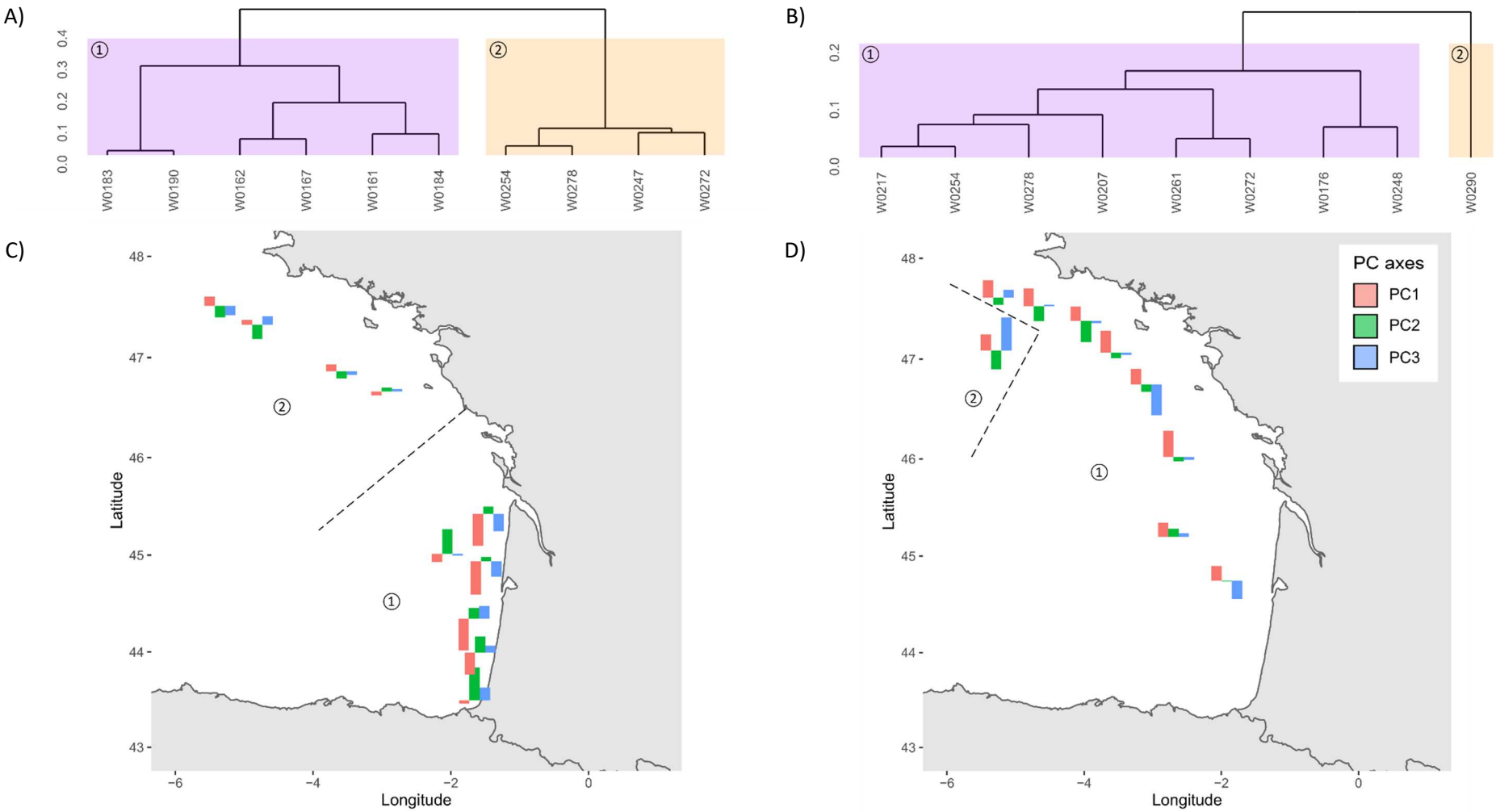


Figure 7. Hierarchical dendrogram showing the clustering of stations (panels A&B) according to the fatty acid profile on the first three principal components (PC) of PCA on the polar lipids and their location in the Bay of Biscay (panels C&D) for ages 1 and 2 sardines (panels A&C) and age 3

421 and older sardines (panels B&D). The numbers indicate the different zones. The PC axes are defined from FATM as: PC1 positive: haptophytes
422 and dinophytes, PC1 negative: diatoms, PC2 positive: not applicable, PC2 negative: copepods and non-diatom phytoplankton, PC3 positive: not
423 applicable, PC3 negative: diatoms.
424

425 **4. Discussion**

426

427 *4.1. Sardine fatty acids profiles in the Bay of Biscay*

428

429 Although we measured and identified more FA, the sardine muscle FA profiles in NL
430 and PL in the BoB were consistent with the composition and proportions of FA obtained in this
431 species in other areas (Bandarra et al., 2018; Biton-Porsmoguer et al., 2020; García-Moreno
432 et al., 2013; Pacetti et al., 2013; Pethybridge et al., 2014). Indeed, the most common FA and
433 the percentages of the different categories of FA in these studies were broadly similar to those
434 that we measured here (e.g. greater proportions of PUFA than SFA and MUFA). We found that
435 the most abundant FA were the 16:0 and 22:6n-3 whatever the lipid fraction considered, but
436 their proportions varied substantially between NL and PL. For instance, the proportions of
437 16:0 and 22:6n-3 are respectively 1.4 and three times higher in PL than in NL. These differences
438 are similar to those reported by Bandarra et al. (1997, 2018), confirming the importance of
439 these two FA as major structural components of fish cell membranes (Sargent et al., 1999).
440 Our EPA/DHA ratios in NL and PL (on average 0.68 and 0.23, respectively) are similar
441 to those found by García-Moreno et al., 2013 (0.60 for sardine oils in spring equivalent to NL),
442 Pethybridge et al., 2014 (0.37 in March and 0.86 in July for NL of sardine muscle) and Bandarra
443 et al., 2018 (0.25 for PL of wild sardine muscle). In our case, the EPA/DHA ratio is three times
444 higher in NL than in PL. This ratio should decrease towards higher trophic levels as DHA is
445 conserved especially in PL and EPA tends to decrease (Dalsgaard et al., 2003; Scott et al.,
446 2002); here, a decline in the ratio is visible with the ageing of sardines, in both lipid fractions.
447 Sardines aged 1 and 2 years have the highest EPA/DHA ratio indicating a more herbivorous

448 diet while those older than 2 years seem to orient their diet towards higher trophic levels such
449 as macrozooplankton.
450
451 *4.2. Ontogenetic and spatial differences in the diet of Bay of Biscay sardines through fatty acids*
452 *profiles*
453
454 In the BoB, the strong spatial distribution pattern in sardines' age reflects the ecology
455 of this species in spring (Bellier et al., 2007; Doray et al., 2018a; Petitgas et al., 2006). Indeed,
456 a majority of young individuals are found near the coast in the Southern part of the BoB (Silva
457 et al., 2009) and near the nutrient rich plumes of the Gironde estuary (Doray, personal
458 communication). Despite this spatially unbalanced age distribution, we found that sardine FA
459 profiles were different depending on age in both NL and PL while accounting for the sampling
460 location. This ontogenetic dietary change in NL is consistent with other studies of sardines'
461 feeding habits based on stomach content (Bachiller and Irigoien, 2015; Garrido et al., 2008a;
462 Le Bourg et al., 2015) and stable isotopes (Bode et al., 2004; Costalago et al., 2012; Le Bourg
463 et al., 2015), which showed that sardines have a more varied diet as they age. According to
464 these studies, smaller and younger sardines have a high proportion of diatoms and copepods
465 (*Microsetella* spp., *Corycaeus* spp.) whereas larger sardines eat more fish eggs, crustacean
466 eggs, and other copepod genera (*Oncaea, Temora, Centropages*). In summary, larger sardines
467 eat more calanoid copepods than smaller ones. This pattern is consistent with our FA profiles
468 that showed a substantial increase in FATM of herbivorous copepods between ages 1 and 3
469 followed by a plateau. In contrast, sardines aged 1 and 2 years show a higher concentration
470 of small prey FATM (e.g. 20:5n-3 for diatoms). Three processes can explain this ontogenetic
471 dietary difference: a morphological change in gills and/or a change in feeding behaviour with

472 age or the overriding effect of prey availability. Indeed, sardines are capable of switching from
473 non-selective filter-feeding to particulate-feeding behaviour (Bachiller et al., 2020; Costalago
474 et al., 2015; Garrido et al., 2008a; Van der Lingen et al., 2009). Van der Lingen et al. (2009)
475 suggested that the filtering apparatus is fully developed when sardines have reached a length
476 of 15 cm, corresponding to *ca.* 48% of the sardines aged 1 year sampled for this study. It has
477 also been suggested that dietary differences in planktivorous pelagic fishes can also be
478 explained by changes in fish feeding behaviour *per se* rather than by morphology (Tanaka et
479 al., 2006). The change in feeding behaviour is supported by the very different FA profiles of
480 young and old sardines sampled in the same station. In addition, the decline in the EPA/DHA
481 ratio with age (meaning more carnivory, Garrido et al., 2008b) may indicate a greater dietary
482 contribution of macrozooplankton in older sardines diet (as DHA accumulates in
483 macrozooplankton; Sargent and Falk-Petersen, 1988; Virtue et al., 2000). However, we do not
484 identify prey for some FA strongly changing with sardines' age (e.g. 15:0) and we lack FA of
485 prey ingested by the particulate-feeding of sardine (e.g. fish eggs, decapods), limiting
486 investigation about macrozooplankton. Nevertheless, prey availability is probably the main
487 driver of the overall change in FA profiles as sardines aged 1 and 2 years are living in the coastal
488 areas of the Southern BoB, where the abundance of primary producers is very high (Huret et
489 al., 2013) while older sardines were sampled in areas near the shelf-break where large
490 copepods are found (Dessier et al., 2018; Petitgas et al., 2018). We lack historical values of
491 sardine FA composition in BoB, but the stronger decline in size-at-age for sardines aged 1 and
492 2 years compared to older ones may reflects changes in primary production that is somehow
493 lagged in secondary production.

494 When we analysed separately sardines aged 1 and 2 years old and older sardines, we
495 found that there was a clear spatial structuring in FA profiles along a geographical gradient. In

496 1 and 2 year old sardine, the proportion of copepods increased towards the North-Western
497 part of the BoB, and the proportion of non-diatom phytoplankton increased in the South-
498 Eastern part of the BoB. The proportion of diatoms was highest near the Gironde estuary and
499 lowest near the Adour river, which is consistent with previous studies about phytoplankton
500 distribution in spring (Marquis et al., 2007). Such spatial structuring was apparent in PL, but
501 was particularly strong in NL which enabled us to identify two additional groups of stations off
502 the Gironde estuary. Nevertheless, there are clear cycles of the primary production with high
503 values some years (2000-2001, 2007-2008) and others with substantially lower concentrations
504 (2003-2005, 2011, 2015; Boëns et al., 2021; Huret et al., 2013). The overlap between primary
505 producers and 1-year-old sardines near the Southern coast of the BoB (Huret et al., 2013;
506 Petitgas et al., 2018) reveals the importance of phytoplankton for younger sardines, although
507 sardines prefer to feed on zooplankton (Garrido et al., 2008a). Young sardines are probably
508 more filter-feeding or consume smaller zooplankton, explaining why sardines aged 1 and 2
509 years do not exhibit such high copepods FATM proportion. For sardines older than 2 years old,
510 we found greater proportions of the FA characteristics of copepods over the entire BoB and
511 the spatial structure was primarily driven by differences in the concentration of diatoms
512 FATM. The general dominance of copepods FATM in the Northern BoB is consistent with past
513 studies that have described the spatial heterogeneity in the hydrobiological characteristics in
514 spring: larger zooplankton are more abundant in the North-Western than in the South-Eastern
515 BoB (Petitgas et al., 2018). Moreover, there is a larger abundance of *Calanus helgolandicus* off
516 the Gironde estuary with particularly high energy density (Dessier et al., 2018). This copepod
517 species is one of the largest in the BoB and may be primarily caught by older sardines (based
518 on stomach contents and trophic levels of the food, Bachiller and Irigoien, 2015; Costalago et
519 al., 2012; Le Bourg et al., 2015). Sardines eat what they can find and suit their feeding mode,

520 which depends on the environment and seasonal phenology of plankton (Costalago et al.,
521 2015; Garrido et al., 2008a; Napolitano et al., 1997). Consequently, the difference in diet of
522 BoB sardines is probably due to food availability along a geographical gradient at a given time
523 and sardines' feeding behaviour may change with their diet as they feed on larger copepods
524 or more macrozooplankton when they age.
525
526 *4.3. Lipid fractions and the ontogenetic variability in fatty acids profiles*
527
528 Our study clearly shows that NL and PL vary differently with endogenous and spatial
529 variables. Indeed, differences in sardine diet were more pronounced when studying NL than
530 PL as has already been shown experimentally (e.g. Bandarra et al., 2018). This reflects the
531 more selective incorporation of some very specific FA into PL than into NL (e.g. n-3 and n-6
532 long-chain PUFA such as EFA), allowing membranes to adapt to changing environmental
533 conditions (Dalsgaard et al., 2003; Soudant et al., 1996). Indeed, the FA profiles of PL undergo
534 a stronger selective incorporation of EFA than that of NL following feeding, retaining
535 preferentially the FA necessary for the functioning of cell membranes (Robin et al., 2003;
536 Szabo et al., 2011). Therefore, PL have a lower nutritional marking compared with NL that,
537 conversely, reflect more tightly feeding variations. In addition, MUFA such as copepods FATM
538 are preferentially oriented to energy storage (Sargent et al., 1999), explaining their greater
539 proportion in NL than in PL. These aspects are important as many recent studies (e.g. Biton-
540 Porsmoguer et al., 2020; Pacetti et al., 2013) rely on TL, which is the sum of NL and PL fractions.
541 As PL/NL ratios can change substantially with fish state, for instance depending on the
542 sampling season or reproductive stage (Bandarra et al., 1997), the use of TL to compare
543 individuals from different species, locations or seasons could be biased and result in

544 ambiguous results. Indeed, the distinction between NL and PL allowed us to better understand
545 the variance in FA composition, which could be largely due to the fact that both lipid fractions
546 reflect different physiological and metabolic processes.

547 The great variability in sardine FA profiles illustrated the importance of testing and
548 accounting for ontogenetic changes in the species of interest, especially when focusing on the
549 NL fraction. Xu et al. (2020) showed that within species *Salmo salar* and *Sparus aurata*, small
550 fish have a faster FA turnover than large fish and hence that age can be a major factor in the
551 rate of FA turnover. Whatever the temporal or spatial comparison of a species FA profile, one
552 should ensure that the fish size and/or age distribution are similar in sampling design or
553 consider appropriate statistical analyses to eliminate potential bias due to the ontogenetic
554 variation in diet. Provided the dynamic nature of NL and PL profiles and their different
555 sensitivity to recent changes in diets, future studies should be careful with the use of the TL
556 extract and we recommend to consider NL and PL fractions separately to deeply investigate
557 the ecological meaning of potential differences in FA profiles.

558

559 *4.4. Limits and perspectives of the study*

560

561 Only some FA reported in the PCA were interpreted due to the limited knowledge of
562 the different FATM and the meaning of the dietary source signature. However, there are other
563 FA that are related to the different PCA axes that we do not really interpret because they are
564 not specifically synthesised by a group of species/trophic level and could not be attributed to
565 particular preys of sardines. Even if copepods and phytoplankton represent the large majority
566 of sardine preys, especially in spring during phytoplankton blooms (Costalago et al., 2012;
567 Dessier et al., 2018; Le Bourg et al., 2015), we may have missed a part of the food

568 characterisation of sardines. Indeed, sardines' stomachs in the BoB do also contain
569 appendicularians and decapods in spring (Bachiller and Irigoien, 2015), for which we could not
570 consider FATM. Thus, developing knowledge about new FATM specific to these groups will be
571 important in a near future to move towards a more global description of sardines' diet with
572 FA. In addition, there is some uncertainty in the proportions of phytoplankton FATM measured
573 in NL as these can be underestimated if they are preferentially incorporated in PL or
574 overestimated as they may be accumulated in copepods that eat phytoplankton (Dalsgaard et
575 al., 2003). Nevertheless, as the spatial structure of the FATM is consistent with that of the
576 distribution of plankton in the BoB (Petitgas et al., 2018), it is very likely that our results are
577 explained by different foraging behaviours (filter-feeding and particulate-feeding).

578 This study can therefore be expanded over time and seasons to determine whether
579 such changes in diet are consistent within and between years and the degree to which the
580 variation in plankton communities affect their FA profiles. Moreover, studying the prey's FA
581 composition could help to better understand the transfer efficiencies of essential FA and the
582 nutrition potential of plankton for small pelagic fish. To this end, a more homogenous
583 sampling with respect to individuals' age over space is needed to disentangle more efficiently
584 the effects of age and geographical location on sardines' FA profiles. However, even if our
585 sampling cover one season and year, the structuration observed with the most abundant FA
586 is consistent with the great diet variability already found with other technics in BoB (Bachiller
587 and Irigoien, 2015; Chouvelon et al., 2014, 2015). This confirms the potential of using FA
588 including some FATM to provide additional information in multi-proxy studies (e.g. Bachiller
589 et al., 2020) and to better understand small pelagic fish population changes.

590

591 **5. Conclusion**

Our study is the first to fully characterise the composition and variability in the FA of sardines in the BoB through NL from PL. There were clear spatial and ontogenetic differences in sardines' FA profiles especially in the NL fraction. Higher total FA contents were observed in the Northern part of the BoB (area of older and larger sardines), with a dominance of FA characterising copepods, while non-diatom phytoplankton FATM prevailed in Southern BoB (area of younger and smaller sardines). Diatoms FATM were highest near the Gironde estuary. This high dependence on younger sardines' diet to phytoplankton in spring suggests that changes in primary production (quantity and quality) may explain the stronger decline in size-at-age of sardines aged 1 and 2 years during the last decade in the BoB. We also showed that it is important to consider both NL and PL fractions as the FA contents of NL are much more variable than those of PL and conversely, PL can provide key information about the long-lasting effects of changes in individuals' diet and environmental conditions. The contribution of FA to trophic studies can clearly enable us to better understand the bottom-up control exerted by the plankton community on the characteristics of small pelagic fish and identify the food-web dynamics of the BoB pelagic ecosystem.

Acknowledgements

We thank Mathieu Doray and Erwan Duhamel, chief survey scientists of PELGAS, for enabling us to collect these data. We are also grateful the crew of R/V 'Thalassa', particularly Hervé Barone and Laurence Pennors for their precious help during the onboard sampling. We are also grateful to two anonymous referees for providing thoughtful comments on the manuscript. PELGAS surveys are co-funded by the European Union through the Data Collection

Framework. P.B. was supported by ISblue project, Interdisciplinary graduate school for the blue planet (ANR-17-EURE-0015) which is co-funded by the French government under the program "Investissements d'Avenir" and by the Regional Council of Brittany (SAD programme).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Ahlgren, G., Vrede, T., Goedkoop, W., 2009. Fatty acid ratios in freshwater fish, zooplankton and zoobenthos – are there specific optima?, in: Lipids in Aquatic Ecosystems. Springer New York, pp. 147–178. https://doi.org/10.1007/978-0-387-89366-2_7

Bachiller, E., Albo-Puigserver, M., Giménez, J., Pennino, M.G., Marí-Mena, N., Esteban, A., Lloret-Lloret, E., Jadaud, A., Carro, B., Bellido, J.M., Coll, M., 2020. A trophic latitudinal gradient revealed in anchovy and sardine from the Western Mediterranean Sea using a multi-proxy approach. Sci. Rep. 10. https://doi.org/10.1038/S41598-020-74602-Y

Bachiller, E., Irigoien, X., 2015. Trophodynamics and diet overlap of small pelagic fish species in the Bay of Biscay. Mar. Ecol. Prog. Ser. 534, 179–198. https://doi.org/10.3354/meps11375

Bandarra, N.M., Batista, I., Nunes, M.L., Empis, J.M., Christie, W.W., 1997. Seasonal changes in lipid composition of sardine (*Sardina pilchardus*). J. Food Sci. 62, 40–42. https://doi.org/10.1111/j.1365-2621.1997.tb04364.x

Bandarra, N.M., Marçalo, A., Cordeiro, A.R., Pousão-Ferreira, P., 2018. Sardine (Sardina pilchardus)

641 lipid composition: does it change after one year in captivity? Food Chem. 244, 408–413.
642 https://doi.org/10.1016/j.foodchem.2017.09.147
643 Bellier, E., Planque, B., Oceanography, P.P.-F., 2007, U., 2007. Historical fluctuations in spawning
644 location of anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) in the Bay of Biscay
645 during 1967–73 and 2000. Wiley Online Libr. 16, 1–15. https://doi.org/10.1111/j.1365-
646 2419.2006.00410.x
647 Benítez-Santana, T., Masuda, R., Juárez Carrillo, E., Ganuza, E., Valencia, A., Hernández-Cruz, C.M.,
648 Izquierdo, M.S., 2007. Dietary n-3 HUFA deficiency induces a reduced visual response in gilthead
649 seabream *Sparus aurata* larvae. Aquaculture 264, 408–417.
650 https://doi.org/10.1016/j.aquaculture.2006.10.024
651 Biton-Porsmoguer, S., Bou, R., Lloret, E., Alcaide, M., Lloret, J., 2020. Fatty acid composition and
652 parasitism of European sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*)
653 populations in the northern Catalan Sea in the context of changing environmental conditions.
654 Conserv. Physiol. 8. https://doi.org/10.1093/conphys/coaa121
655 Bode, A., Alvarez-Ossorio, M.T., Carrera, P., Lorenzo, J., 2004. Reconstruction of trophic pathways
656 between plankton and the North Iberian sardine (*Sardina pilchardus*) using stable isotopes. Sci.
657 Mar. 68, 165–178.
658 Boëns, A., Grellier, P., Lebigre, C., Petitgas, P., 2021. Determinants of growth and selective mortality in
659 anchovy and sardine in the Bay of Biscay. Fish. Res. 239.
660 https://doi.org/10.1016/j.fishres.2021.105947
661 Brosset, P., Fromentin, J.M., Ménard, F., Pernet, F., Bourdeix, J.H., Bigot, J.L., Van Beveren, E., Pérez
662 Roda, M.A., Choy, S., Saraux, C., 2015a. Measurement and analysis of small pelagic fish condition:
663 a suitable method for rapid evaluation in the field. J. Exp. Mar. Bio. Ecol. 462, 90–97.
664 https://doi.org/10.1016/j.jembe.2014.10.016
665 Brosset, P., Fromentin, J.M., Van Beveren, E., Lloret, J., Marques, V., Basilone, G., Bonanno, A., Carpi,
666 P., Donato, F., Čikeš Keč, V., De Felice, A., Ferreri, R., Gašparević, D., Giráldez, A., Gücü, A., Iglesias,

667 M., Leonori, I., Palomera, I., Somarakis, S., Tičina, V., Torres, P., Ventero, A., Zorica, B., Ménard,
668 F., Saraux, C., 2017. Spatio-temporal patterns and environmental controls of small pelagic fish
669 body condition from contrasted Mediterranean areas. Prog. Oceanogr. 151, 149–162.
670 https://doi.org/10.1016/j.pocean.2016.12.002
671 Brosset, P., Le Bourg, B., Costalago, D., BĂnaru, D., Van Beveren, E., Bourdeix, J.H., Fromentin, J.M.,
672 Ménard, F., Saraux, C., 2016. Linking small pelagic dietary shifts with ecosystem changes in the
673 Gulf of Lions. Mar. Ecol. Prog. Ser. 554, 157–171. https://doi.org/10.3354/meps11796
674 Brosset, P., Ménard, F., Fromentin, J.M., Bonhommeau, S., Ulses, C., Bourdeix, J.H., Bigot, J.L., Van
675 Beveren, E., Roos, D., Saraux, C., 2015b. Influence of environmental variability and age on the
676 body condition of small pelagic fish in the Gulf of Lions. Mar. Ecol. Prog. Ser. 529, 219–231.
677 https://doi.org/10.3354/meps11275
678 Budge, S.M., Devred, E., Forget, M.H., Stuart, V., Trzcinski, M.K., Sathyendranath, S., Platt, T., 2014.
679 Estimating concentrations of essential omega-3 fatty acids in the ocean: supply and demand. ICES
680 J. Mar. Sci. 71, 1885–1893. https://doi.org/10.1093/icesjms/fsu003
681 Budge, S.M., Iverson, S.J., Koopman, H.N., 2006. Studying trophic ecology in marine ecosystems using
682 fatty acids: a primer on analysis and interpretation. Mar. Mammal Sci. 22, 759–801.
683 https://doi.org/10.1111/j.1748-7692.2006.00079.x
684 Cañavate, J.P., 2019. Advancing assessment of marine phytoplankton community structure and
685 nutritional value from fatty acid profiles of cultured microalgae. Rev. Aquac.
686 https://doi.org/10.1111/raq.12244
687 Carozza, D.A., Bianchi, D., Galbraith, E.D., 2019. Metabolic impacts of climate change on marine
688 ecosystems: implications for fish communities and fisheries. Glob. Ecol. Biogeogr. 28, 158–169.
689 https://doi.org/10.1111/geb.12832
690 Cartes, J.E., 2011. Temporal changes in lipid biomarkers, especially fatty acids, of the deep-sea
691 crustaceans *Boreomysis arctica* and *Nematoscelis megalops*: implications of their biological cycle
692 and habitat near the seabed. J. Mar. Biol. Assoc. United Kingdom 91, 783–792.

https://doi.org/10.1017/S0025315410002018

Certain, G., Masse, J., Van Canneyt, O., Petitgas, P., Doremus, G., Santos, M.B., Ridoux, V., 2011. Investigating the coupling between small pelagic fish and marine top predators using data collected from ecosystem-based surveys. Mar. Ecol. Prog. Ser. 422, 23–39. https://doi.org/10.3354/meps08932

Chouvelon, T., Chappuis, A., Bustamante, P., Lefebvre, S., Mornet, F., Guillou, G., Violamer, L., Dupuy, C., 2014. Trophic ecology of European sardine Sardina pilchardus and European anchovy Engraulis encrasicolus in the Bay of Biscay (north-east Atlantic) inferred from $\delta^{13}C$ and $\delta^{15}N$ values of fish and identified mesozooplanktonic organisms. J. Sea Res. 85, 277–291. https://doi.org/10.1016/j.seares.2013.05.011

Chouvelon, T., Dessier, A., Bustamante, P., Mornet, F., Pignon-Mussaud, C., Dupuy, C., 2015. Small pelagic fish feeding patterns in relation to food resource variability: an isotopic investigation for *Sardina pilchardus* and *Engraulis encrasicolus* from the Bay of Biscay (north-east Atlantic). Mar. Biol. 162, 15–37. https://doi.org/10.1007/s00227-014-2577-5

Costalago, D., Garrido, S., Palomera, I., 2015. Comparison of the feeding apparatus and diet of European sardines *Sardina pilchardus* of Atlantic and Mediterranean waters: ecological implications. J. Fish Biol. 86, 1348–1362. https://doi.org/10.1111/jfb.12645

Costalago, D., Navarro, J., Álvarez-Calleja, I., Palomera, I., 2012. Ontogenetic and seasonal changes in the feeding habits and trophic levels of two small pelagic fish species. Mar. Ecol. Prog. Ser. 460, 169–181. https://doi.org/10.3354/meps09751

Couturier, L.I.E., Michel, L.N., Amaro, T., Budge, S.M., da Costa, E., De Troch, M., Di Dato, V., Fink, P., Giraldo, C., Le Grand, F., Loaiza, I., Mathieu-Resuge, M., Nichols, P.D., Parrish, C.C., Sardenne, F., Vagner, M., Pernet, F., Soudant, P., 2020. State of art and best practices for fatty acid analysis in aquatic sciences. ICES J. Mar. Sci. 77, 2375–2395. https://doi.org/10.1093/icesjms/fsaa121

Cury, P., Bakun, A., Crawford, R.J.M., Jarre, A., Quiñones, R.A., Shannon, L.J., Verheye, H.M., 2000. Small pelagics in upwelling systems: patterns of interaction and structural changes in “wasp-

719 waist" ecosystems, in: ICES Journal of Marine Science. pp. 603–618.
720 https://doi.org/10.1006/jmsc.2000.0712
721 Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers
722 in the pelagic marine environment. Adv. Mar. Biol. https://doi.org/10.1016/S0065-
723 2881(03)46005-7
724 Dessier, A., Bustamante, P., Chouvelon, T., Huret, M., Pagano, M., Marquis, E., Rousseaux, F., Pignon-
725 Mussaud, C., Mornet, F., Bréret, M., Dupuy, C., 2018. The spring mesozooplankton variability and
726 its relationship with hydrobiological structure over year-to-year changes (2003–2013) in the
727 southern Bay of Biscay (Northeast Atlantic). Prog. Oceanogr. 166, 76–87.
728 https://doi.org/10.1016/j.pocean.2018.04.011
729 Doray, M., Hervy, C., Huret, M., Petitgas, P., 2018a. Spring habitats of small pelagic fish communities
730 in the Bay of Biscay. Prog. Oceanogr. 166, 88–108.
731 https://doi.org/10.1016/J.POCEAN.2017.11.003
732 Doray, M., Petitgas, P., Huret, M., Duhamel, E., Romagnan, J.B., Authier, M., Dupuy, C., Spitz, J., 2018b.
733 Monitoring small pelagic fish in the Bay of Biscay ecosystem, using indicators from an integrated
734 survey. Prog. Oceanogr. 166, 168–188. https://doi.org/10.1016/j.pocean.2017.12.004
735 García-Moreno, P.J., Pérez-Gálvez, R., Morales-Medina, R., Guadix, A., Guadix, E.M., 2013. Discarded
736 species in the west Mediterranean sea as sources of omega-3 PUFA. Eur. J. Lipid Sci. Technol. 115,
737 982–989. https://doi.org/10.1002/ejlt.201300021
738 Garrido, S., Ben-Hamadou, R., Oliveira, P.B., Cunha, M.E., Chícharo, M.A., Van Der Lingen, C.D., 2008a.
739 Diet and feeding intensity of sardine *Sardina pilchardus*: correlation with satellite-derived
740 chlorophyll data. Mar. Ecol. Prog. Ser. 354, 245–256. https://doi.org/10.3354/meps07201
741 Garrido, S., Rosa, R., Ben-Hamadou, R., Cunha, M.E., Chícharo, M.A., Van Der Lingen, C.D., 2008b.
742 Spatio-temporal variability in fatty acid trophic biomarkers in stomach contents and muscle of
743 Iberian sardine (*Sardina pilchardus*) and its relationship with spawning. Mar. Biol. 154, 1053–
744 1065. https://doi.org/10.1007/s00227-008-0999-7

745 Gatti, P., Petitgas, P., Huret, M., 2017. Comparing biological traits of anchovy and sardine in the Bay of
746 Biscay: a modelling approach with the Dynamic Energy Budget. Ecol. Modell. 348, 93–109.
747 https://doi.org/10.1016/j.ecolmodel.2016.12.018
748 Graeve, M., Greenacre, M.J., 2020. The selection and analysis of fatty acid ratios: a new approach for
749 the univariate and multivariate analysis of fatty acid trophic markers in marine pelagic organisms.
750 Limnol. Oceanogr. Methods 18, 196–210. https://doi.org/10.1002/lom3.10360
751 Hauss, H., Franz, J.M.S., Sommer, U., 2012. Changes in N:P stoichiometry influence taxonomic
752 composition and nutritional quality of phytoplankton in the Peruvian upwelling. J. Sea Res. 73,
753 74–85. https://doi.org/10.1016/j.seares.2012.06.010
754 Hixson, S.M., Arts, M.T., 2016. Climate warming is predicted to reduce omega-3, long-chain,
755 polyunsaturated fatty acid production in phytoplankton. Glob. Chang. Biol. 22, 2744–2755.
756 https://doi.org/10.1111/gcb.13295
757 Hulbert, A.J., Kelly, M.A., Abbott, S.K., 2014. Polyunsaturated fats, membrane lipids and animal
758 longevity. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. https://doi.org/10.1007/s00360-
759 013-0786-8
760 Huret, M., Sourisseau, M., Petitgas, P., Struski, C., Léger, F., Lazure, P., 2013. A multi-decadal hindcast
761 of a physical-biogeochemical model and derived oceanographic indices in the Bay of Biscay. J.
762 Mar. Syst. 109–110, S77–S94. https://doi.org/10.1016/j.jmarsys.2012.02.009
763 ICES, 2019. Sardine (*Sardina pilchardus*) in divisions 8.a–b and 8.d (Bay of Biscay). Rep. ICES Advis.
764 Committee, 2019. ICES Advice 2019, pil.27.8abd.
765 ICES, 2011. Report of the Workshop on Age Reading of European Atlantic Sardine (WKARAS), 14-18
766 February 2011, Lisbon, Portugal. ICES C. 2011/ACOM42 91.
767 Izquierdo, M.S., 1996. Essential fatty acid requirements of cultured marine fish larvae. Aquac. Nutr.
768 https://doi.org/10.1111/j.1365-2095.1996.tb00058.x
769 Jónasdóttir, S.H., 2019. Fatty acid profiles and production in marine phytoplankton. Mar. Drugs.
770 https://doi.org/10.3390/md17030151

771 Kattner, G., Hagen, W., 1995. Polar herbivorous copepods - different pathways in lipid biosynthesis.
772 ICES J. Mar. Sci. 52, 329–335. https://doi.org/10.1016/1054-3139(95)80048-4
773 Koven, W.M., Tandler, A., Kissil, G.W., Sklan, D., Friezlander, O., Harel, M., 1990. The effect of dietary
774 (n-3) polyunsaturated fatty acids on growth, survival and swim bladder development in *Sparus*
775 *aurata* larvae. Aquaculture 91, 131–141. https://doi.org/10.1016/0044-8486(90)90182-M
776 Le Bourg, B., Bănaru, D., Saraux, C., Nowaczyk, A., Le Luherne, E., Jadaud, A., Bigot, J.L., Richard, P.,
777 2015. Trophic niche overlap of sprat and commercial small pelagic teleosts in the Gulf of Lions
778 (NW Mediterranean Sea). J. Sea Res. 103, 138–146.
779 https://doi.org/10.1016/j.seares.2015.06.011
780 Le Grand, F., Kraffe, E., Marty, Y., Donaghy, L., Soudant, P., 2011. Membrane phospholipid composition
781 of hemocytes in the Pacific oyster *Crassostrea gigas* and the Manila clam *Ruditapes*
782 *philippinarum*. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 159, 383–391.
783 https://doi.org/10.1016/j.cbpa.2011.04.006
784 Legendre, P., De Cáceres, M., 2013. Beta diversity as the variance of community data: dissimilarity
785 coefficients and partitioning. Wiley Online Libr. 16, 951–963. https://doi.org/10.1111/ele.12141
786 Lloret, J., Shulman, G., Love, R.M., 2013. Condition and health indicators of exploited marine fishes.
787 https://doi.org/10.1002/9781118752777
788 Marquis, E., Niquil, N., Delmas, D., Hartmann, H.J., Bonnet, D., Carlotti, F., Herbland, A., Labry, C.,
789 Sautour, B., Laborde, P., Vézina, A., Dupuy, C., 2007. Inverse analysis of the planktonic food web
790 dynamics related to phytoplankton bloom development on the continental shelf of the Bay of
791 Biscay, French coast. Estuar. Coast. Shelf Sci. 73, 223–235.
792 https://doi.org/10.1016/j.ecss.2007.01.003
793 Martino, J.C., Fowler, A.J., Doubleday, E.A., Grammer, G.L., Gillanders, B.M., 2019. Using otolith
794 chronologies to understand long-term trends and extrinsic drivers of growth in fisheries. Wiley
795 Online Libr. 10. https://doi.org/10.1002/ecs2.2553
796 Marty, Y., Delaunay, F., Moal, J., Samain, J.F., 1992. Changes in the fatty acid composition of *Pecten*

maximus (L.) during larval development. J. Exp. Mar. Bio. Ecol. 163, 221–234. https://doi.org/10.1016/0022-0981(92)90051-B

Masuda, R., Takeuchi, T., Tsukamoto, K., Sato, H., Shimizu, K., Imaizumi, K., 1999. Incorporation of dietary docosahexaenoic acid into the central nervous system of the yellowtail *Seriola quinqueradiata*. Brain. Behav. Evol. 53, 173–179. https://doi.org/10.1159/000006592

Mathieu-Resuge, M., Kraffe, E., Le Grand, F., Boens, A., Bideau, A., Lluch-Cota, S.E., Racotta, I.S., Schaal, G., 2019. Trophic ecology of suspension-feeding bivalves inhabiting a north-eastern Pacific coastal lagoon: comparison of different biomarkers. Mar. Environ. Res. 145, 155–163. https://doi.org/10.1016/j.marenvres.2019.02.016

Meyer, L., Pethybridge, H., Nichols, P.D., Beckmann, C., Huveneers, C., 2019. Abiotic and biotic drivers of fatty acid tracers in ecology: a global analysis of chondrichthyan profiles. Funct. Ecol. 33, 1243–1255. https://doi.org/10.1111/1365-2435.13328

Napolitano, G.E., Pollero, R.J., Gayoso, A.M., MacDonald, B.A., Thompson, R.J., 1997. Fatty acids as trophic markers of phytoplankton blooms in the Bahia Blanca estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). Biochem. Syst. Ecol. 25, 739–755. https://doi.org/10.1016/S0305-1978(97)00053-7

Nunes, C., Silva, A., Marques, V., Ganias, K., 2011. Integrating fish size, condition, and population demography in the estimation of Atlantic sardine annual fecundity. Ciencias Mar. 37, 565–584.

Olsen, R.E., Henderson, R.J., 1989. The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. J. Exp. Mar. Bio. Ecol. 129, 189–197. https://doi.org/10.1016/0022-0981(89)90056-7

Pacetti, D., Balzano, M., Colella, S., Santojanni, A., Frega, N.G., 2013. Effect of spawning on furan fatty acid profile of edible muscle and organ tissues from sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*). J. Agric. Food Chem. 61, 3969–3977. https://doi.org/10.1021/jf400555u

Pethybridge, H., Bodin, N., Arsenault-Pernet, E.J., Bourdeix, J.H., Brisset, B., Bigot, J.L., Roos, D., Peter, M., 2014. Temporal and inter-specific variations in forage fish feeding conditions in the NW

Mediterranean: lipid content and fatty acid compositional changes. Mar. Ecol. Prog. Ser. 512, 39–54. https://doi.org/10.3354/meps10864

Pethybridge, H.R., Parrish, C.C., Morrongiello, J., Young, J.W., Farley, J.H., Gunasekera, R.M., Nichols, P.D., 2015. Spatial patterns and temperature predictions of tuna fatty acids: tracing essential nutrients and changes in primary producers. PLoS One 10. https://doi.org/10.1371/journal.pone.0131598

Petitgas, P., Huret, M., Dupuy, C., Spitz, J., Authier, M., Romagnan, J.B., Doray, M., 2018. Ecosystem spatial structure revealed by integrated survey data. Prog. Oceanogr. 166, 189–198. https://doi.org/10.1016/j.pocean.2017.09.012

Petitgas, P., Masse, J., Bourriau, P., Beillois, P., Bergeron, J.P., Delmas, D., Herbland, A., Koueta, N., Froidefond, J.M., Santos, M., 2006. Hydro-plankton characteristics and their relationship with sardine and anchovy distributions on the French shelf of the Bay of Biscay. Sci. Mar. 70S1, 161–172.

Pigliucci, M., 2005. Evolution of phenotypic plasticity: where are we going now? Trends Ecol. Evol. 20, 481–486. https://doi.org/10.1016/j.tree.2005.06.001

R Core Team, 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Remize, M., Planchon, F., Loh, A.N., Le Grand, F., Bideau, A., Le Goic, N., Fleury, E., Miner, P., Corvaisier, R., Volety, A., Soudant, P., 2020. Study of synthesis pathways of the essential polyunsaturated fatty acid 20:5n-3 in the diatom Chaetoceros muelleri using ^{13}C-isotope labeling. Biomolecules 10. https://doi.org/10.3390/biom10050797

Riquelme-Bugueño, R., Pantoja-Gutiérrez, S., Jorquera, E., Anabalón, V., Srain, B., Schneider, W., 2020. Fatty acid composition in the endemic Humboldt Current krill, *Euphausia mucronata* (*Crustacea, Euphausiacea*) in relation to the phytoplankton community and oceanographic variability off Dichato coast in central Chile. Prog. Oceanogr. 188. https://doi.org/10.1016/j.pocean.2020.102425

Robin, J.H., Regost, C., Arzel, J., Kaushik, S.J., 2003. Fatty acid profile of fish following a change in dietary fatty acid source: model of fatty acid composition with a dilution hypothesis. Aquaculture 225, 283–293. https://doi.org/10.1016/S0044-8486(03)00296-5

Rosa, R., Gonzalez, L., Broitman, B.R., Garrido, S., Santos, A.M.P., Nunes, M.L., 2010. Bioenergetics of small pelagic fishes in upwelling systems: relationship between fish condition, coastal ecosystem dynamics and fisheries. Mar. Ecol. Prog. Ser. 410, 205–218. https://doi.org/10.3354/meps08635

Saraux, C., Van Beveren, E., Brosset, P., Queiros, Q., Bourdeix, J.H., Dutto, G., Gasset, E., Jac, C., Bonhommeau, S., Fromentin, J.M., 2019. Small pelagic fish dynamics: a review of mechanisms in the Gulf of Lions. Deep. Res. Part II Top. Stud. Oceanogr. 159, 52–61. https://doi.org/10.1016/j.dsr2.2018.02.010

Sargent, J., 1978. Marine wax esters. Sci. Prog. 65, 437–458.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., Tocher, D., 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture. https://doi.org/10.1016/S0044-8486(99)00191-X

Sargent, J.R., Falk-Petersen, S., 1988. The lipid biochemistry of calanoid copepods, in: Biology of Copepods. Springer Netherlands, pp. 101–114. https://doi.org/10.1007/978-94-009-3103-9_9

Sargent, J.R., Falk-Petersen, S., 1981. Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: lipids and fatty acids in *Meganyctiphanes norvegica*, *Thysanoessa raschi* and *T. inermis* during mid-winter. Mar. Biol. 62, 131–137. https://doi.org/10.1007/BF00388175

Sargent, J.R., McEvoy, L.A., Bell, J.G., 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds, in: Aquaculture. pp. 117–127. https://doi.org/10.1016/S0044-8486(97)00122-1

Scott, C.L., Kwasniewski, S., Falk-Petersen, S., Sargent, J.R., 2002. Species differences, origins and functions of fatty alcohols and fatty acids in the wax esters and phospholipids of *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* from Arctic waters. Mar. Ecol. Prog. Ser. 235, 127–

134. https://doi.org/10.3354/meps235127

Silva, A., Skagen, D.W., Uriarte, A., Massé, J., Santos, M.B., Marques, V., Carrera, P., Beillois, P., Pestana, G., Porteiro, C., Stratoudakis, Y., 2009. Geographic variability of sardine dynamics in the Iberian Biscay region. ICES J. Mar. Sci. 66, 495–508. https://doi.org/10.1093/icesjms/fsn225

Soudant, P., Marty, Y., Moal, J., Robert, R., Quéré, C., Le Coz, J.R., Samain, J.F., 1996. Effect of food fatty acid and sterol quality on *Pecten maximus* gonad composition and reproduction process. Aquaculture 143, 361–378. https://doi.org/10.1016/0044-8486(96)01276-8

Stearns, S., 1992. *The evolution of life histories*. No. 575 S81.

Szabo, A., Mézes, M., Hancz, C., Molnár, T., Varga, D., Romvári, R., Fébel, H., 2011. Incorporation dynamics of dietary vegetable oil fatty acids into the triacylglycerols and phospholipids of tilapia (*Oreochromis niloticus*) tissues (fillet, liver, visceral fat and gonads). Aquac. Nutr. 17. https://doi.org/10.1111/j.1365-2095.2009.00743.x

Tanaka, H., Aoki, I., Ohshimo, S., 2006. Feeding habits and gill raker morphology of three planktivorous pelagic fish species off the coast of northern and western Kyushu in summer. J. Fish Biol. 68, 1041–1061. https://doi.org/10.1111/j.0022-1112.2006.00988.x

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. https://doi.org/10.1080/713610925

Turchini, G.M., Torstensen, B.E., Ng, W.K., 2009. Fish oil replacement in finfish nutrition. Rev. Aquac. https://doi.org/10.1111/j.1753-5131.2008.01001.x

Van Beveren, E., Bonhommeau, S., Fromentin, J.M., Bigot, J.L., Bourdeix, J.H., Brosset, P., Roos, D., Saraux, C., 2014. Rapid changes in growth, condition, size and age of small pelagic fish in the Mediterranean. Mar. Biol. 161, 1809–1822. https://doi.org/10.1007/s00227-014-2463-1

Van der Lingen, C., Bertrand, A., Bode, A., Brodeur, R., Cubillos, L., Espinoza, P., Friedland, K., Garrido, S., Irigoien, X., Miller, T., Möllmann, C., Rodriguez-Sanchez, R., Tanaka, H., Temming, A., 2009. Trophic dynamics of small pelagic fish, in: Center Marine Environnemental Studies. p. 31.

Véron, M., Duhamel, E., Bertignac, M., Pawlowski, L., Huret, M., 2020a. Major changes in sardine

growth and body condition in the Bay of Biscay between 2003 and 2016: temporal trends and drivers. Prog. Oceanogr. 182, 102274. https://doi.org/10.1016/j.pocean.2020.102274

Véron, M., Duhamel, E., Bertignac, M., Pawlowski, L., Huret, M., Baulier, L., 2020b. Determinism of temporal variability in size at maturation of sardine *Sardina pilchardus* in the Bay of Biscay. Front. Mar. Sci. 7. https://doi.org/10.3389/fmars.2020.567841

Virtue, P., Mayzaud, P., Albessard, E., Nichols, P., 2000. Use of fatty acids as dietary indicators in northern krill, *Meganyctiphanes norvegica*, from northeastern Atlantic, Kattegat, and Mediterranean waters. Can. J. Fish. Aquat. Sci. 57, 104–114. https://doi.org/10.1139/f00-182

Xu, H., Turchini, G., Francis, D., Liang, M., Mock, T., Rombenso, A., Ai, Q., 2020. Are fish what they eat? A fatty acid's perspective. Prog. Lipid Res. 101064.

Zwolinski, J., Stratoudakis, Y., Sares, E., 2001. Intra-annual variation in the batch fecundity of sardine off Portugal. J. Fish Biol. 58, 1633–1645. https://doi.org/10.1111/j.1095-8649.2001.tb02318.x