



HAL
open science

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

► **To cite this version:**

Mathilde Bertrand, Pablo Brosset, Philippe Soudant, Christophe Lebigre. Spatial and ontogenetic variations in sardine feeding conditions in the Bay of Biscay through fatty acid composition. *Marine Environmental Research*, 2022, 173, 105514, 12 p. 10.1016/j.marenvres.2021.105514 . hal-03485223

HAL Id: hal-03485223

<https://hal.univ-brest.fr/hal-03485223>

Submitted on 5 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

3
4 **Mathilde Bertrand^{1,2*}, Pablo Brosset^{1,2,3}, Philippe Soudant², Christophe Lebigre^{1*}**

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

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

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

10
11 ***Corresponding author:**

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

13 Ifremer, Laboratoire de Biologie Halieutique,
14 ZI Pointe du Diable - CS 10070, 29 280 Plouzané, France

15
16 **Abstract**

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

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

34

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

36 1. Introduction

37

38 Food characteristics are one of the most important biotic factors influencing
39 individuals' fitness as it allows animals to extract energy from their environment. This energy
40 is then allocated to life history traits and any alteration in its quantity or quality leads to a
41 decrease in survival, growth, and reproductive success (Pigliucci, 2005; Stearns, 1992).
42 Furthermore, the environment governs communities' composition and dynamic by controlling
43 the abundance and assemblage of primary producers and their consumers (Dalsgaard et al.,
44 2003; Hauss et al., 2012). For instance, Hixson and Arts (2016) and Pethybridge et al. (2015)
45 predicted a decrease in the nutritional quality of phytoplankton along increasing sea surface
46 temperature, potentially (i) affecting the recruitment and dynamics of forage fish (and their
47 predators), and (ii) having consequences for human food safety (Budge et al., 2014) since fish
48 may become less abundant and/or less nutritious. In other words, changes at the base of
49 trophic chains can strongly influence the fitness of all species in the upper trophic levels,
50 making it crucial to understand as such changes may have important consequences for stock
51 productivity, ecosystem dynamics and fisheries yields (Carozza et al., 2019; Martino et al.,
52 2019).

53 The European sardine (*Sardina pilchardus*) plays a central role in the transfer of energy
54 between planktonic compartments and higher trophic levels in pelagic food webs (Certain et
55 al., 2011; Cury et al., 2000). In the Bay of Biscay (BoB), there has been a marked decrease in
56 size-at-age, weight-at-age and body condition of sardine (Doray et al., 2018b; Véron et al.,
57 2020a, 2020b), a pattern similar in another small pelagic fish at the same trophic level, the
58 European anchovy (*Engraulis encrasicolus*, Doray et al., 2018b). More specifically between
59 2000 and 2015, mean length of sardine decreased particularly at age 1 fish (ca. from 18.5 to

60 15 cm, i.e. 20%) and body condition declined by 15% on average across all ages (Doray et al.,
61 2018b; Véron et al., 2020a). However, their size-at-maturity (*ca.* 14 cm) did not change and
62 their age-at-maturity remains at 1 year in the BoB (Véron et al., 2020b). These declines have
63 started while the stock had a relatively low exploitation rate and the increase in harvest was
64 unrelated to the decline in fish growth and selective mortality (Boëns et al., 2021), making it
65 unlikely that the recent decline in morphometry of sardines in this area can be attributed to
66 fisheries-induced evolution. Therefore, such a decline in size-at-age and body condition is
67 more likely to be driven by environmental changes in the BoB, though the actual mechanism
68 remains elusive (Véron et al., 2020a, 2020b). Moreover, these phenotypic trends are similar
69 to those observed in the Gulf of Lions since 2008, in which food quantity and quality have
70 been identified as the main drivers leading to the decline of sardines size and body condition
71 and the subsequent collapse of its fishery (Brosset et al., 2017, 2015b; Saraux et al., 2019). As
72 sardine's stock of the BoB sustains major French and Spanish fisheries (totalling 32,299 tons
73 landed in 2018; ICES, 2019), it is important to determine whether bottom-up processes also
74 explain the decline in size-at-age and body condition in this species.

75 A bottom-up control of the phenotypic characteristics of sardines may result from
76 changes in their diet: a decrease in the quantity of food available per individual due to density-
77 dependent competition (the survival rate of juveniles increases in this stock; Doray et al.,
78 2018b; Van Beveren et al., 2014), and/or a decrease in the quality of food (as it seems to be
79 the case with sardines of the Gulf of Lions; Bachiller et al., 2020; Brosset et al., 2016; Saraux
80 et al., 2019). Sardines primarily feed on small species of zooplankton (copepods, decapods,
81 cirripedes, fish eggs and cladocerans) and phytoplankton (diatoms and dinoflagellates) but
82 whose contribution to individuals' diet varies depending on fish length, season and region
83 considered (Costalago et al., 2015; Garrido et al., 2008a; Van der Lingen et al., 2009). Indeed,

84 during sardine spawning periods (October to May, with peaks in November and April; [Gatti et](#)
85 [al., 2017](#)), their broad distribution appears fragmented by the presence of cold bottom water
86 ([Bellier et al., 2007](#)). Thus, in Spring the biomass of sardines is higher along the coast in the
87 Southern BoB, near the Loire estuary, and in the waters South-West of Brittany ([Doray et al.,](#)
88 [2018a](#)). In general, this population structure coincides with the distribution of sardine eggs
89 ([Bellier et al., 2007](#); [Petitgas et al., 2006](#)) and younger sardines are usually located in the
90 Southern BoB ([Silva et al., 2009](#)). In the BoB, the abundance of primary producers and
91 zooplankton is also strongly structured spatially as the amount of chlorophyll-a is particularly
92 high near the coasts and estuaries (Adour, Gironde, Loire; [Huret et al., 2013](#)) and large
93 copepods are particularly abundant near the shelf-break of the BoB ([Dessier et al., 2018](#)).
94 Studies based on stable isotopes and stomach contents showed that sardines' diet changed
95 as they aged (especially in spring and summer; [Bachiller and Irigoien, 2015](#); [Costalago et al.,](#)
96 [2012](#); [Le Bourg et al., 2015](#)) and that there was no significant spatial pattern in fish diet within
97 the BoB ([Chouvelon et al., 2014](#)). However, the characterisation of lipids and their variation
98 may allow us to learn more about the sardines' diet and the constraints exerted by the
99 modification of planktonic communities on their biology. Indeed, lipids are a source of energy
100 (neutral lipids, hereafter NL) and underpin the properties of cell membranes (polar lipids,
101 hereafter PL; [Hulbert et al., 2014](#); [Tocher, 2003](#)). Lipids comprise saturated and unsaturated
102 carbon chains called fatty acids (FA) that are particularly useful biomarkers of organisms' diet
103 ([Cartes, 2011](#); [Dalsgaard et al., 2003](#); [Meyer et al., 2019](#); [Riquelme-Bugueño et al., 2020](#)).
104 Indeed, the FA synthesis chains differ between zoo- and phytoplankton meaning that the
105 presence or absence of some FA can reflect changes in sardines' diet ([Graeve and Greenacre,](#)
106 [2020](#)). Thus, FA have been used as qualitative and semi-quantitative food web biomarkers and
107 have proven to be a valuable method to define food web relationships, trophic positioning,

108 and the dietary behaviours of marine species (Turchini et al., 2009; Xu et al., 2020). FA can
109 also inform us about the reproductive status of fish as NL are strongly solicited during
110 reproduction (Gatti et al., 2017; Rosa et al., 2010) and largest sardines may have a longer
111 laying period due to their larger quantities of NL (Nunes et al., 2011; Zwolinski et al., 2001).
112 Furthermore, marine fish must find essential fatty acids (EFA; e.g. n-3 and n-6 polyunsaturated
113 FA at 20 and 22 carbons) in their food as they have little or no ability to synthesise them *de*
114 *novo* (Ahlgren et al., 2009; Hulbert et al., 2014; Sargent et al., 1999). It has been shown that
115 EFA deficiencies can affect many vital functions such as growth, survival, stress resistance, and
116 immune system (Benítez-Santana et al., 2007; Izquierdo, 1996; Koven et al., 1990). Therefore,
117 EFA composition and quantity can enable us to test if fish nutritional needs are fully satisfied,
118 both qualitatively and quantitatively (Sargent et al., 1997). Consequently, the variation in
119 sardines' FA composition and concentration reflects their food characteristics (Bandarra et al.,
120 1997) and their phenology (growth or reproduction; Pacetti et al., 2013), making these
121 markers invaluable to examine whether bottom-up processes are acting on the phenotype of
122 this species.

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

132

133 **2. Materials and methods**

134

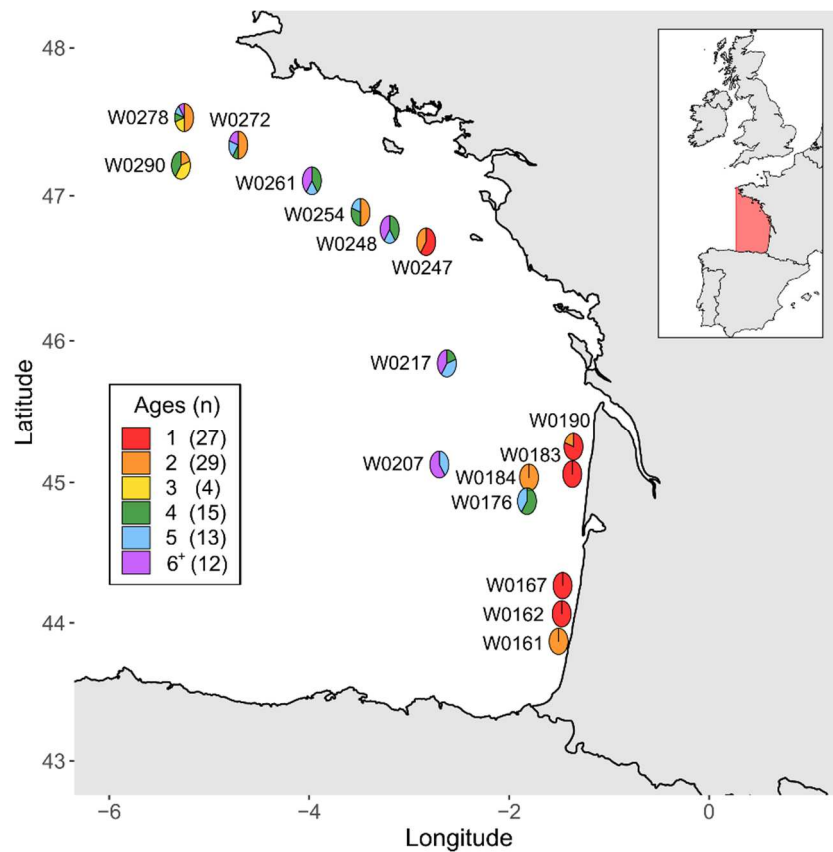
135 *2.1. Acquisition of sardine samples: PELGAS 2018*

136

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

156 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).
 157 In addition, a piece of muscle was taken from each selected sardine and stored in the freezer
 158 at -80°C until lipid analysis in the laboratory. Overall, we collected 100 individual muscle
 159 samples during the 2018 survey.

160



161

162 Figure 1. Spatial distribution of sardines sampled in the Bay of Biscay, as part of PELGAS 2018.

163 Colours of the pie charts indicate the age of fish sampled for this study (with 6+ individuals
 164 aged 6 years and over), and "n" is the number of individuals at each age.

165

166 Table 1. Geographical coordinates and biological parameters of sardines at the 16 sampling

167 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

168

169 *2.2. Grinding, lipid extraction and storage*

170

171 Before any manipulation, the glassware was heated to 450°C for 6 hours and the metal
172 or Teflon materials were rinsed with acetone to avoid contamination of the samples. We first
173 solidified muscle samples in liquid nitrogen and passed them through a ball mill (1 min at 30
174 oscillations/sec). We retrieved between 200 and 250 mg of shred for each sample and added
175 6 mL of a CHCl₃/MeOH mixture (2/1, v/v) to extract lipids ([Mathieu-Resuge et al., 2019](#)). We
176 then vigorously shook vials to re-suspend the shred and improve lipids' extraction. Prior to
177 their storage at -20°C, the samples were passed under a flow of N₂, shaken, placed in an
178 ultrasonic bath for 10 min, and agitated for at least 20 min.

179

180 *2.3. Analyses of lipid classes*

181

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

188

189 *2.4. Fatty acid composition*

190

191 To determine the composition of FA, lipid extracts stored at -20°C were first shaken for
192 20 min, centrifuged for 15 min at 3,000 rpm and 1 mL of each sample was transferred to new
193 vials and evaporated to dryness. Dry lipid extracts were then re-suspended three times with
194 500 µL of CHCl₃/MeOH (98/2; v/v) and gently deposited on top of a silica micro-column ([Marty
195 et al., 1992](#)). We eluted NL with 10 mL CHCl₃/MeOH (98/2; v/v) in a 22 mL vial and PL with 20
196 mL MeOH in another 22 mL vial ([Le Grand et al., 2011](#)). Upstream, an internal standard
197 composed of 20 µL of C23:0 (0.115 µg/µL) has been added to each 22 mL vial. After the elution,
198 the NL and PL fractions were evaporated to dryness using a Genevac centrifugal evaporator
199 (program: Low Boiling Point; temperature: 30°C).

200 After evaporation, the dry NL and PL fractions were re-suspended three times with 500
201 µL CHCl₃/MeOH (2/1; v/v) and transferred into 7 mL vials. These were evaporated dry under
202 N₂ flow and we then added 800 µL of H₂SO₄/MeOH (3.4%) and incubated the samples for 10
203 min at 100°C after vortexing ([Budge et al., 2006](#); [Le Grand et al., 2011](#)). Heat and sulphuric
204 acid catalyse the cleavage of ester bonds and methanol provided the CH₃ groups for the
205 formation of FA methyl esters (FAME). We extracted FAME by adding 800 µL of hexane and

206 1.5 mL of hexane-saturated distilled water, and by shaking and centrifuging the NL and PL
207 fractions 1 min at 1,000 rpm. FAME solubilise in hexane while the catalyst and glycerol mix
208 with water, resulting in two phases. We discarded the denser aqueous phase and repeated
209 this step twice by adding only 1.5 mL of hexane-saturated distilled water. Finally, the samples
210 were delicately placed in the freezer at -20°C without removing the aqueous phase. After
211 several hours, we quickly transferred the unfrozen upper organic phase into 2 mL vials, which
212 are flushed with N₂ and stored in a refrigerator prior analysis.

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

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

$$225 \quad M_{FA,i} = \frac{A_{FA,i} \times M_{C23:0}}{A_{C23:0}} \quad (1) \qquad P_{FA,i} = \frac{100 \times M_{FA,i}}{\sum_{i=1}^n M_{FA,i}} \quad (2)$$

226 with M_{FA} the mass of a given FA, A_{FA} its area on the chromatogram, $A_{C23:0}$ the area of the
227 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 $\mu\text{g}/\text{mg}$ 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

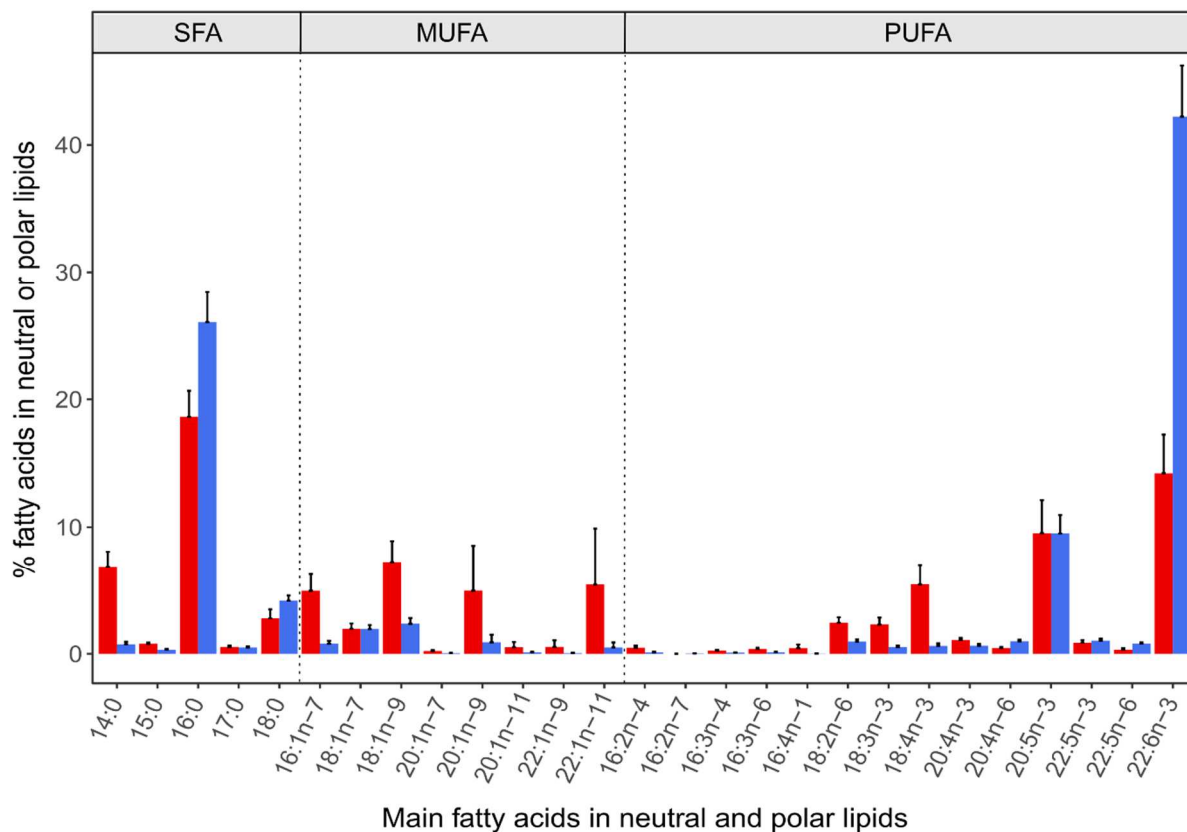
288 *3.1. Sardine fatty acids profiles in the Bay of Biscay*

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

312 variables explained more variance than endogenous variables in PL (18 and 14% respectively,
313 Table 2). While sardines' weight, sex and maturity stage explained a very limited amount of
314 variance in FA, individuals' age explained nearly 20% of the total variation in FA (Table 2, Figure
315 3). More specifically, the increase in individuals' age is associated with major changes in the
316 content of some FA (Figure 4 and Figure 5). In NL, we observed an increase in FA specific of
317 copepods (20:1n-9 and 22:1n-11, Figure 3A and Figures 4B&C) and a decline in FA specific of
318 phytoplankton (20:5n-3, i.e. diatoms, Figure 3A and Figure 4D). For other FA non-associated
319 with specific prey groups, there were increases (e.g. 20:1n-11, Figure 4A) and declines with
320 sardines' age (e.g. 15:0, 18:4n-3, Figures 4E&F). Some of these FA also changed similarly in PL
321 (Figure 3B), which was particularly evident for the increase in 20:1n-9 (i.e. copepods, Figure
322 4H) and the decrease in 20:5n-3 (i.e. diatoms, Figure 4I) with sardines' age. As for NL, an
323 increase (20:4n-3, Figure 4G) and a decline (15:0, iso15:0, Figure 4J&K) with sardines' age were
324 observed for other FA non-associated with specific prey groups. We found a decrease in the
325 EPA/DHA ratio with age in NL and PL fractions, although the ratio was three times greater in
326 NL (Figure 5). Noteworthy, changes seemed to occur primarily between the sardines aged 1
327 and 2 and those older than 2 years old, both in NL and PL fractions (Figure 4 and Figure 5).

328

329

330

331

332

333

334

335

336 Table 2. Summary of the redundancy analyses quantifying the relationship between
 337 endogenous variables and fatty acid composition of sardines (i.e. constrained effect) after
 338 accounting for the contribution of geographic effects (i.e. conditioned effect). The percentage
 339 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 (λ_1)	6.44 (18.9)	4.71 (13.9)
Unconstrained (λ_2)	20.63 (60.6)	23.21 (68.3)
Variable	λ_1 / λ_2	λ_1 / λ_2
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)

340

341

342

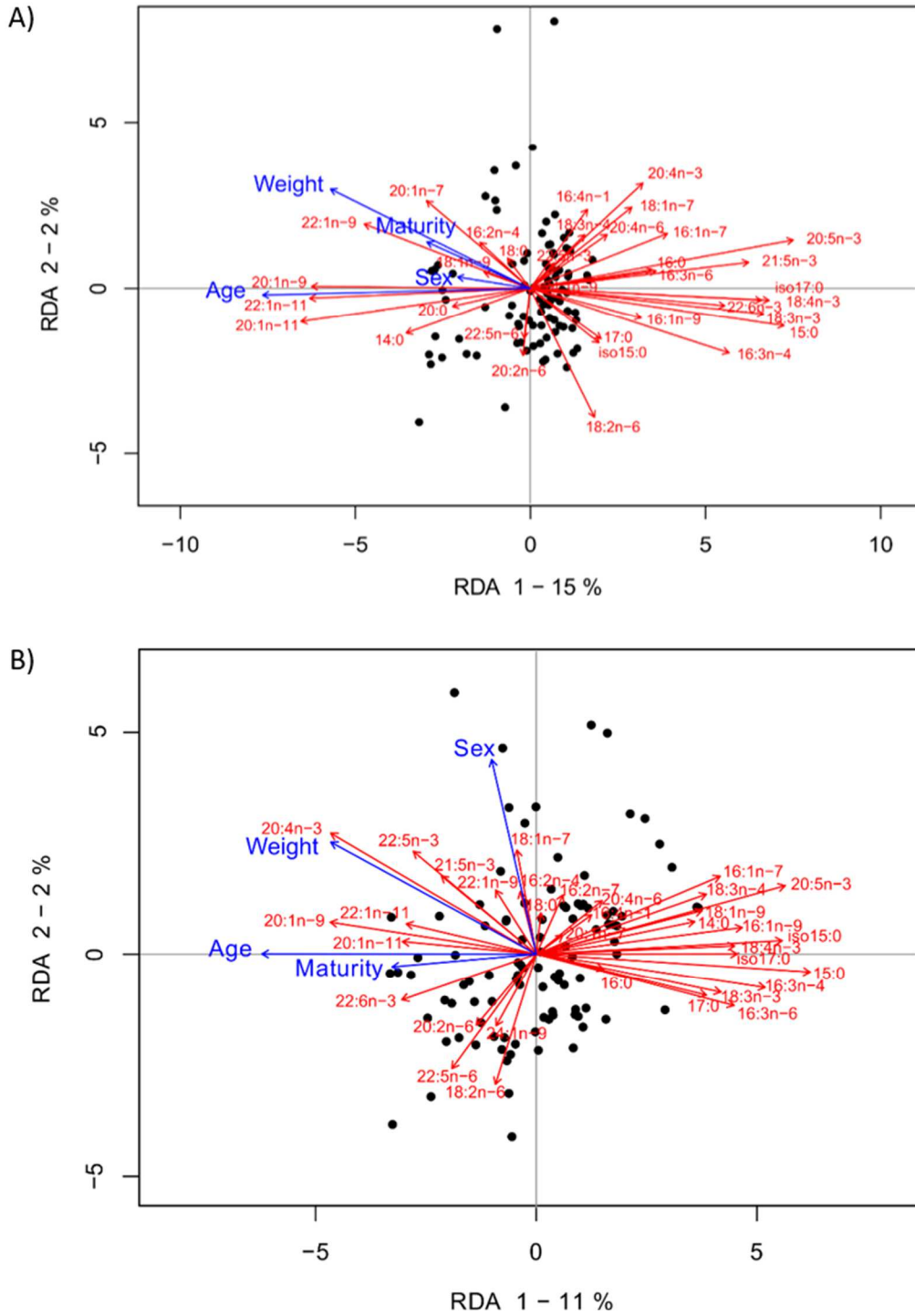
343

344

345

346

347

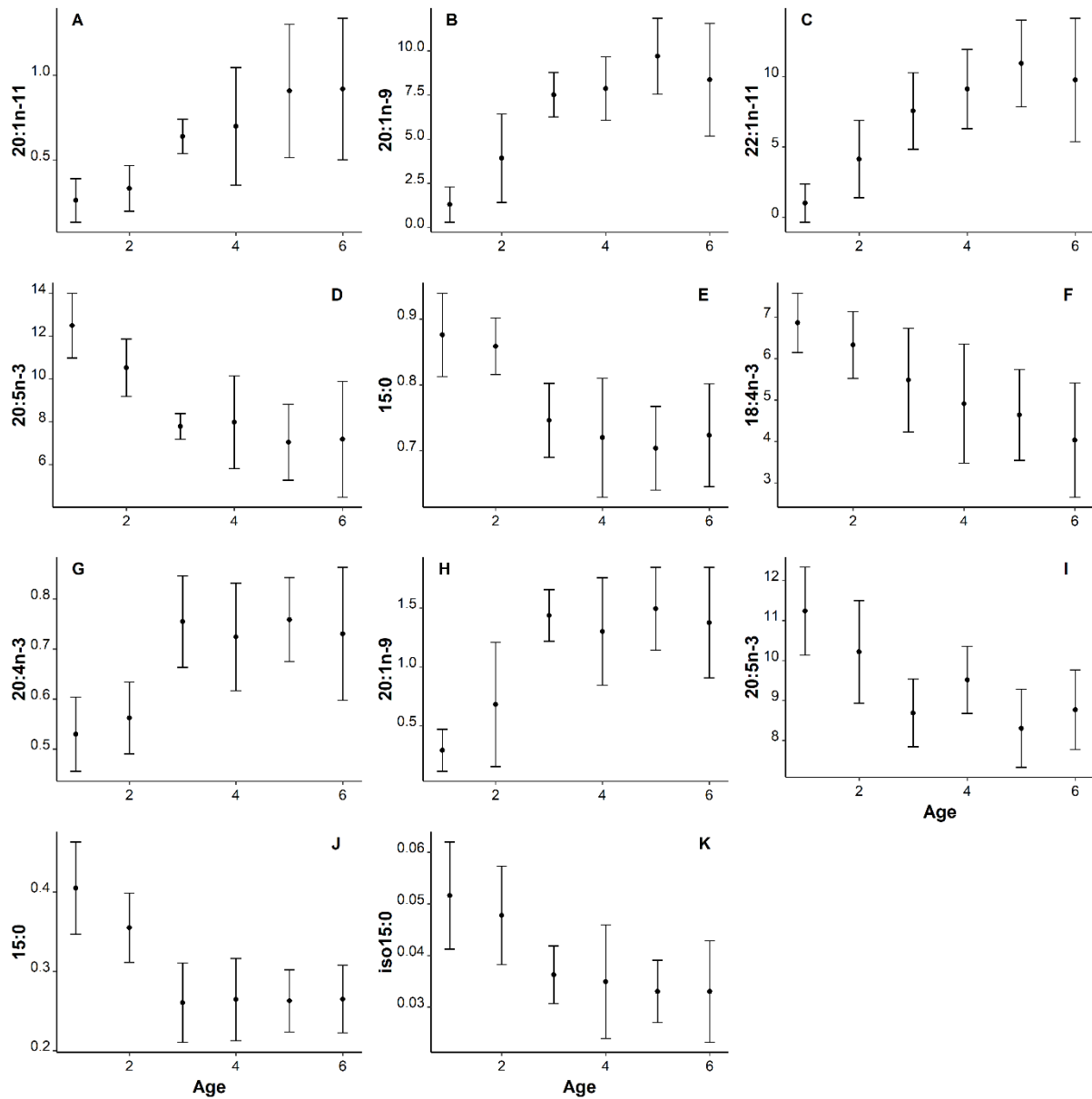


348

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



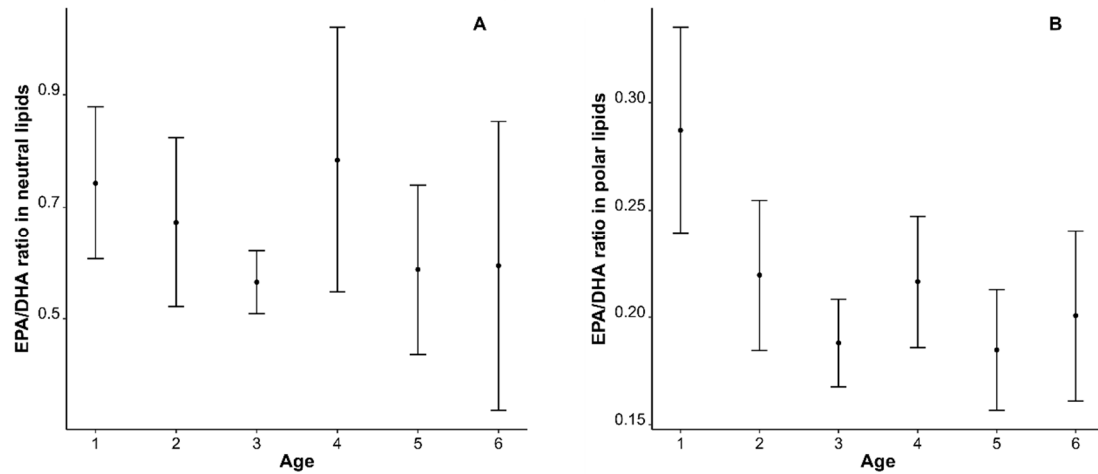
354

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



359

360 Figure 5. Changes in the EPA/DHA ratio according to the age of sardines for neutral lipids

361 (panel A) and for polar lipids (panel B). Age is provided in years.

362

363 3.3. Spatial variability of fatty acids profiles

364

365 To determine whether there was a spatial structure in the 35 main FA, we first carried

366 out PCA to produce integrative values of FA profiles for each individual. For NL, the first axis

367 of the PCA (PC1_NL) was positively related to 20:1n-9 and 22:1n-11 (copepods FATM) and

368 negatively related to 22:6n-3 and iso17:0 (non-diatom phytoplankton and bacterial FATM,

369 respectively; Figure S1A and Table S2). The second axis (PC2_NL) was positively related to a

370 mix of 20:5n-3, 18:3n-3 and 18:4n-3 (diatoms and non-diatom phytoplankton FATM) and

371 negatively related to 22:5n-6 (non-diatom phytoplankton FATM mainly present in

372 *Prymnesiophyceae*, *Pavlovophyceae*, *Pelagophyceae* and *Raphidophyceae*; Figure S1A and

373 Table S2). The third axis (PC3_NL) was positively related to 16:4n-1, 16:1n-7 and 16:2n-4

374 (FATM typical of diatoms, Figure S1B and Table S2). For PL, the first axis of the PCA (PC1_PL)

375 was negatively related to 16:1n-7, 16:3n-4 and 20:5n-3 (diatoms FATM) and positively related

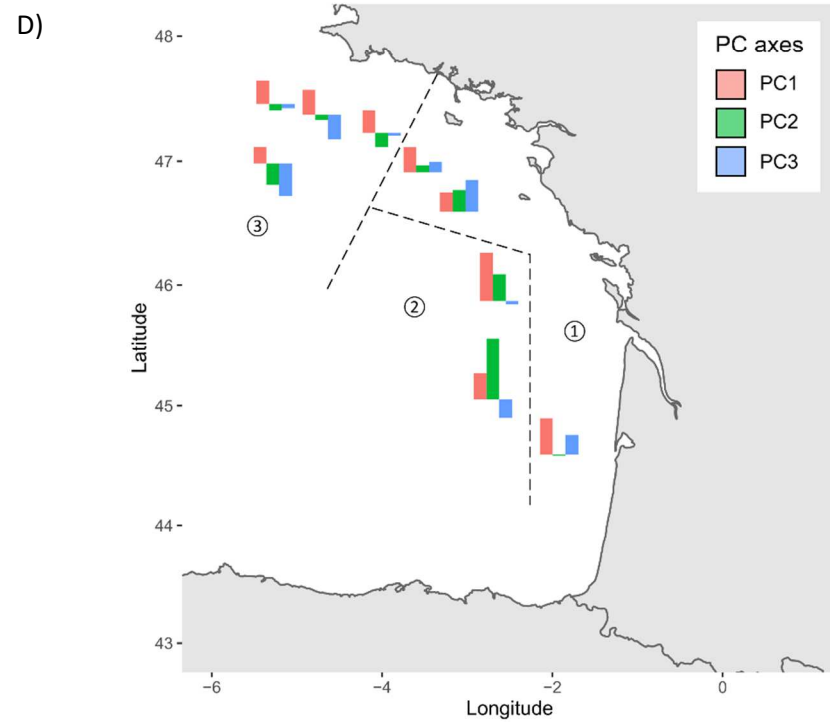
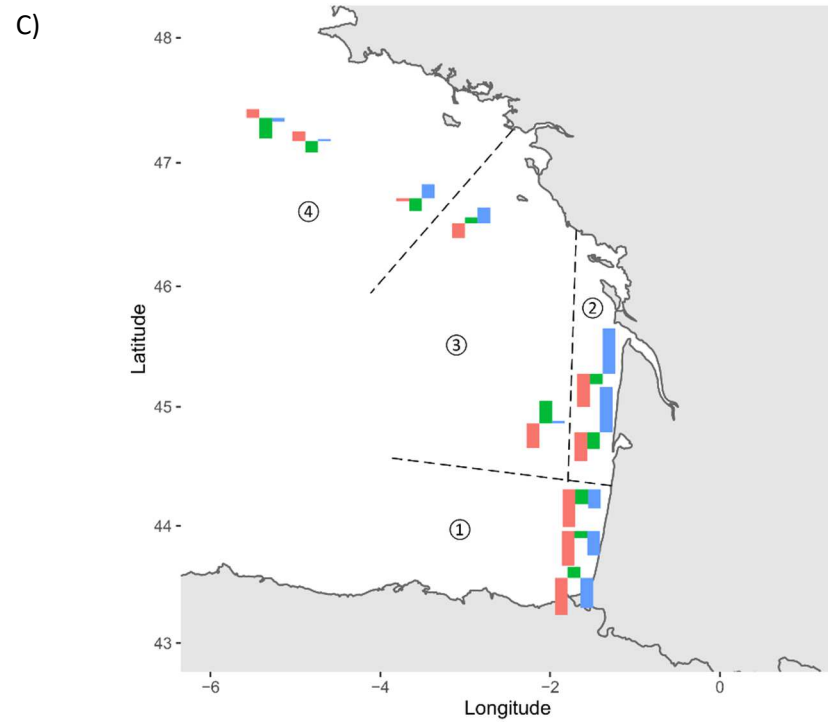
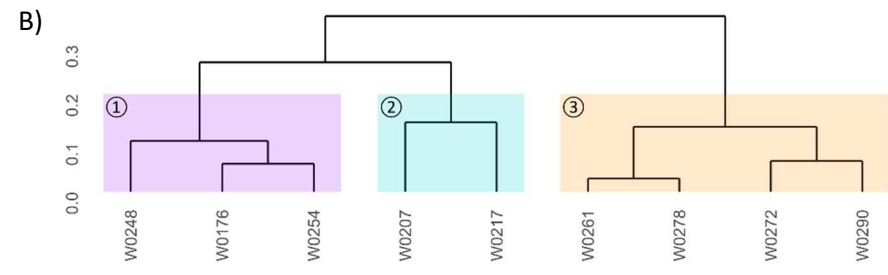
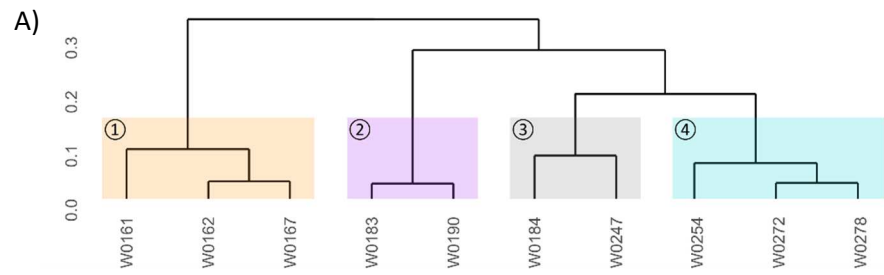
376 to 22:6n-3 (abundant in haptophytes and dinophytes, Figure S1C and Table S2), while the

377 second axis (PC2_PL) was negatively related to 20:1n-9 and 22:1n-11 (FATM of copepods) and
378 18:2n-6, 18:3n-3 and 18:4n-3 (non-diatom phytoplankton FATM, Figure S1C and Table S2).
379 Finally, the third axis (PC3_PL) was negatively related to the diatoms FATM (i.e. 16:2n-7 and
380 16:4n-1) that were poorly explained by PC1_PL (Figure S1D, Table S2).

381 To account for the effect of ontogenetic diet changes in FA profiles, we carried out two
382 separate clustering analyses (based on the values of the first three PCA axes) for NL and PL:
383 one with only ages 1 and 2 sardines (10 stations) and another with older sardines (9 stations).
384 For NL in 1 and 2 years old sardines, the clustering analysis identified four clusters of stations
385 across the BoB (Figure 6A) distributed along a latitudinal gradient (Figure 6C). More
386 specifically, the Southern group was characterised by primarily negative values of the PC1_NL
387 (i.e. more non-diatom phytoplankton and bacterial FATM) and negative values of PC3_NL (i.e.
388 less diatoms FATM; Figure 6C, area 1). The Northern group was characterised by positive
389 values of PC1_NL indicating more FATM of copepods (Figure 6C, area 4). The third and fourth
390 groups, located in between Southern and Northern groups, showed slightly negative and
391 positive values for PC1_NL and PC3_NL, respectively, indicating both low concentrations of
392 copepods FATM and more FATM of diatoms (Figure 6C, areas 2 and 3). The group located near
393 the coast had particularly high PC3_NL (i.e. more diatoms 16-carbon FATM; Figure 6C, area 2)
394 compared with the group further from the coast which was also characterised by positive
395 values of PC2_NL (i.e. a mix of diatoms and non-diatom phytoplankton FATM; Figure 6C, area
396 3). We found a very similar spatial structure in sardines older than 2 years (Figures 6B&D) with
397 the notable difference that PC1_NL values were all positive (depicting a large part of copepods
398 FATM, Figure 6D). Stations located near the Gironde estuary were characterised by strong
399 positive values of PC1_NL and PC2_NL (i.e. more copepods and a mix of diatoms and non-
400 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).

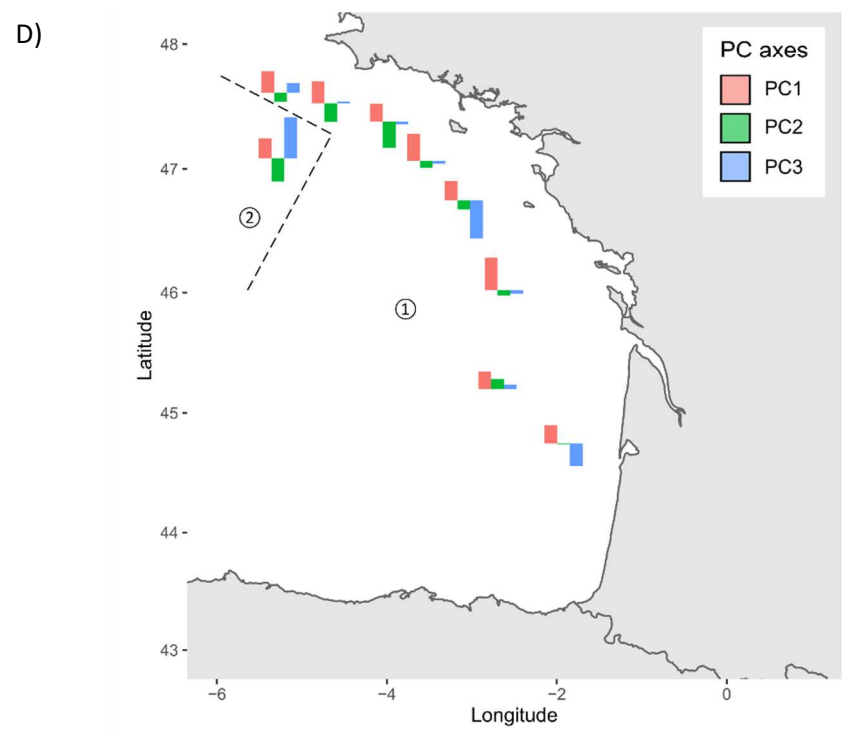
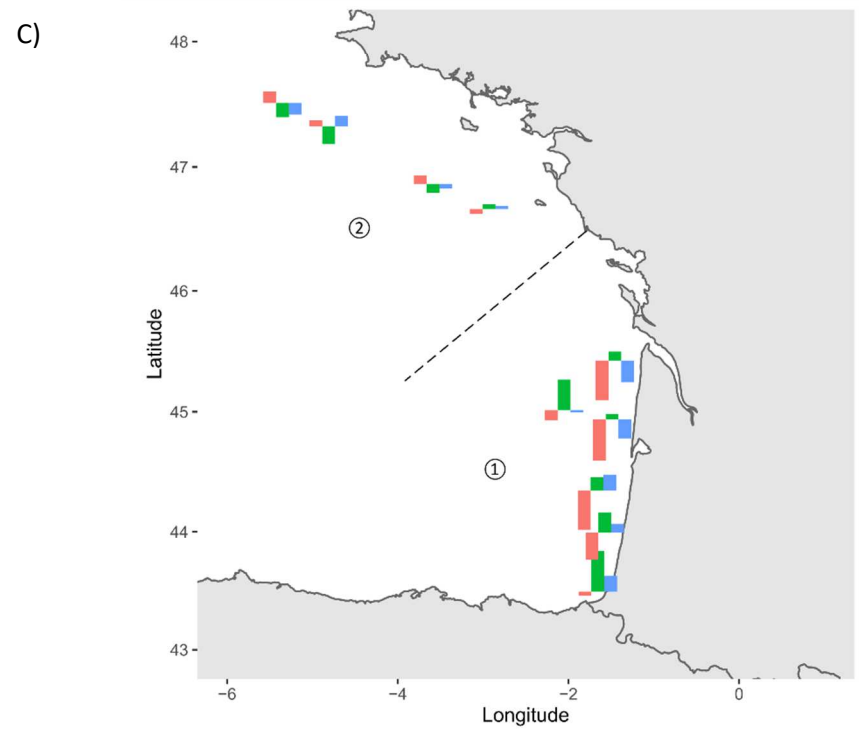
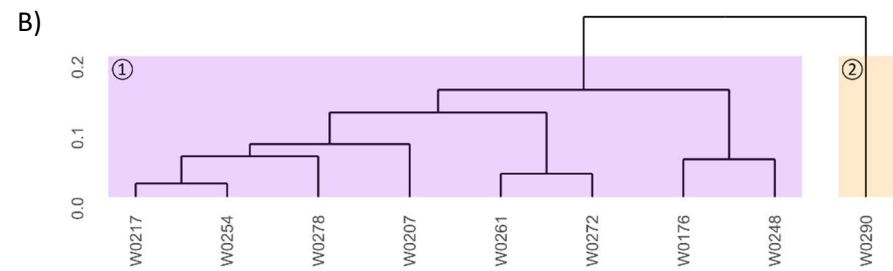
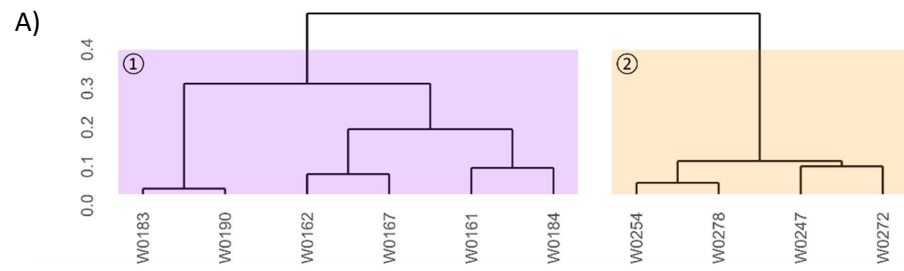
405 For PL, the hierarchical dendrogram for 1 and 2 years old sardines based on PCA axes
406 values distinguished two clusters (Figures 7A&C). Southern stations had negative and positive
407 values of PC1_PL and PC2_PL, respectively, indicating FA characteristic of diatoms (Figure 7C,
408 area 1). Northern 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

415 3 and older sardines (panels B&D). The numbers indicate the different zones. The PC axes are defined from FATM as: PC1 positive: copepods,
416 PC1 negative: non-diatom phytoplankton and bacteria, PC2 positive: diatoms and non-diatom phytoplankton, PC2 negative: non-diatom
417 phytoplankton, PC3 positive: diatoms, PC3 negative: not applicable.



418

419 Figure 7. Hierarchical dendrogram showing the clustering of stations (panels A&B) according to the fatty acid profile on the first three principal
 420 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 reflect 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; Chauvelon 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**

592

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

608

609 **Acknowledgements**

610

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

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

620

621 **Declaration of competing interest**

622

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

625

626 **References**

627

628 Ahlgren, G., Vrede, T., Goedkoop, W., 2009. Fatty acid ratios in freshwater fish, zooplankton and
629 zoobenthos – are there specific optima?, in: *Lipids in Aquatic Ecosystems*. Springer New York, pp.
630 147–178. https://doi.org/10.1007/978-0-387-89366-2_7

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

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

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

640 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-](https://doi.org/10.1111/j.1365-2419.2006.00410.x)
646 [2419.2006.00410.x](https://doi.org/10.1111/j.1365-2419.2006.00410.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., Sarau, 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., BĂnar, 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.

693 <https://doi.org/10.1017/S0025315410002018>

694 Certain, G., Masse, J., Van Canneyt, O., Petitgas, P., Doremus, G., Santos, M.B., Ridoux, V., 2011.

695 Investigating the coupling between small pelagic fish and marine top predators using data

696 collected from ecosystem-based surveys. *Mar. Ecol. Prog. Ser.* 422, 23–39.

697 <https://doi.org/10.3354/meps08932>

698 Chouvelon, T., Chappuis, A., Bustamante, P., Lefebvre, S., Mornet, F., Guillou, G., Violamer, L., Dupuy,

699 C., 2014. Trophic ecology of European sardine *Sardina pilchardus* and European anchovy

700 *Engraulis encrasicolus* in the Bay of Biscay (north-east Atlantic) inferred from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values

701 of fish and identified mesozooplanktonic organisms. *J. Sea Res.* 85, 277–291.

702 <https://doi.org/10.1016/j.seares.2013.05.011>

703 Chouvelon, T., Dessier, A., Bustamante, P., Mornet, F., Pignon-Mussaïd, C., Dupuy, C., 2015. Small

704 pelagic fish feeding patterns in relation to food resource variability: an isotopic investigation for

705 *Sardina pilchardus* and *Engraulis encrasicolus* from the Bay of Biscay (north-east Atlantic). *Mar.*

706 *Biol.* 162, 15–37. <https://doi.org/10.1007/s00227-014-2577-5>

707 Costalago, D., Garrido, S., Palomera, I., 2015. Comparison of the feeding apparatus and diet of

708 European sardines *Sardina pilchardus* of Atlantic and Mediterranean waters: ecological

709 implications. *J. Fish Biol.* 86, 1348–1362. <https://doi.org/10.1111/jfb.12645>

710 Costalago, D., Navarro, J., Álvarez-Calleja, I., Palomera, I., 2012. Ontogenetic and seasonal changes in

711 the feeding habits and trophic levels of two small pelagic fish species. *Mar. Ecol. Prog. Ser.* 460,

712 169–181. <https://doi.org/10.3354/meps09751>

713 Couturier, L.I.E., Michel, L.N., Amaro, T., Budge, S.M., da Costa, E., De Troch, M., Di Dato, V., Fink, P.,

714 Giraldo, C., Le Grand, F., Loaiza, I., Mathieu-Resuge, M., Nichols, P.D., Parrish, C.C., Sardenne, F.,

715 Vagner, M., Pernet, F., Soudant, P., 2020. State of art and best practices for fatty acid analysis in

716 aquatic sciences. *ICES J. Mar. Sci.* 77, 2375–2395. <https://doi.org/10.1093/icesjms/fsaa121>

717 Cury, P., Bakun, A., Crawford, R.J.M., Jarre, A., Quiñones, R.A., Shannon, L.J., Verheye, H.M., 2000.

718 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-](https://doi.org/10.1016/S0065-2881(03)46005-7)
723 [2881\(03\)46005-7](https://doi.org/10.1016/S0065-2881(03)46005-7)

724 Dessier, A., Bustamante, P., Chauvelon, 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-](https://doi.org/10.1007/s00360-013-0786-8)
759 [013-0786-8](https://doi.org/10.1007/s00360-013-0786-8)

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](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](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*

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

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

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

806 Meyer, L., Pethybridge, H., Nichols, P.D., Beckmann, C., Huveneers, C., 2019. Abiotic and biotic drivers
807 of fatty acid tracers in ecology: a global analysis of chondrichthyan profiles. Funct. Ecol. 33, 1243–
808 1255. <https://doi.org/10.1111/1365-2435.13328>

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

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

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

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

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

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

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

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

832 Petitgas, P., Masse, J., Bourriau, P., Beillois, P., Bergeron, J.P., Delmas, D., Herbland, A., Koueta, N.,
833 Froidefond, J.M., Santos, M., 2006. Hydro-plankton characteristics and their relationship with
834 sardine and anchovy distributions on the French shelf of the Bay of Biscay. Sci. Mar. 70S1, 161–
835 172.

836 Pigliucci, M., 2005. Evolution of phenotypic plasticity: where are we going now? Trends Ecol. Evol. 20,
837 481–486. <https://doi.org/10.1016/j.tree.2005.06.001>

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

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

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

849 Robin, J.H., Regost, C., Arzel, J., Kaushik, S.J., 2003. Fatty acid profile of fish following a change in dietary
850 fatty acid source: model of fatty acid composition with a dilution hypothesis. *Aquaculture* 225,
851 283–293. [https://doi.org/10.1016/S0044-8486\(03\)00296-5](https://doi.org/10.1016/S0044-8486(03)00296-5)

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

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

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

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

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

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

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

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

875 134. <https://doi.org/10.3354/meps235127>

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

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

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

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

887 Tanaka, H., Aoki, I., Ohshimo, S., 2006. Feeding habits and gill raker morphology of three planktivorous
888 pelagic fish species off the coast of northern and western Kyushu in summer. *J. Fish Biol.* 68,
889 1041–1061. <https://doi.org/10.1111/j.0022-1112.2006.00988.x>

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

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

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

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

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

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

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

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

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

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

913