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Spatial changes in fatty acids signatures of the great scallop *Pecten maximus* across the Bay of Biscay continental shelf

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ABSTRACT

The spatial variability of food resources along continental margins can strongly influence the physiology and ecology of benthic bivalves. We explored the variability of food sources of the great scallop *Pecten maximus*, by determining their fatty acid (FA) composition along an inshore–offshore gradient in the Bay of Biscay (from 15 to 190 m depth). The FA composition of the digestive gland showed strong differences between shallow and deep-water habitats. This trend was mainly driven by their content in diatom-characteristic fatty acids, which are abundant near the coast. Scallops collected from the middle of the continental shelf were characterized by higher contents of flagellate markers than scallops from shallow habitats. This could be related to a permanent vertical stratification in the water column, which reduced vertical mixing of waters, thereby enhancing organic matter recycling through the microbial loop. In the deeper water station (190 m), FA compositions were close to the compositions found in scallops from shallow areas, which suggest that scallops could have access to the same resources (*i.e.* diatoms). Muscle FA composition was more indicative of the physiological state of scallops over this depth range, revealing contrasting reproductive strategies among the two coastal sites and metabolic or physiological adaptation at greater depth (*e.g.* structural and functional adjustments of membrane composition). This study therefore revealed contrasted patterns between shallow and deeper habitats for both *P. maximus* muscle and digestive gland tissues. This emphasizes the variability in the diet of this species along its distribution range, and stresses the importance of analyzing different tissues for their FA composition in order to better understand their physiology and ecology.

1. Introduction

Spatial distribution of *Pecten maximus* along the eastern coast of the Northern Atlantic Ocean depends on environmental variables, such as temperature, salinity and food availability (Brand et al., 2006). On continental shelves, nutrient concentrations and irradiance are influenced by physical factors (Guillaud et al., 2008; Puillat et al., 2004) that impact the spatial variability of marine primary producers (Beaufort and Heussner, 2001; Morin et al., 1991). This variability may further lead to spatial differences in the diet of suspension-feeders in these ecosystems (Koutsikopoulos and LeCann, 1996). The diet of *P. maximus* is mainly consisted of phytoplankton but can also include dissolved organic carbon (Roditi et al., 2000), resuspended sediment (Raikow and Hamilton, 2001), benthic algae, microheterotrophs and bacteria (Kang et al., 1999; Kreeger and Newell, 2001; Lorrain et al., 2002; Nichols and Garling, 2000). However, while the assessment of large spatial availabilities of organic matter sources is limited by their short term occurrences and requires extensive sampling, it is possible to measure different chemical components such as fatty acids that can document the nature of food sources assimilated by marine consumers, in consumers tissues (Mayzaud et al., 1989; Canuel et al., 1995).

Analysis of fatty acid compositions in animal tissues is now widely used to identify food sources in marine organisms (Budge et al., 2006; Dalsgaard et al., 2003; Kelly and Scheibling, 2012), including macrozoobenthic species such as polychaetes

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; BFA, branched fatty acids; PUFA, polyunsaturated fatty acids; TFA, total fatty acids; DHA, docosahexaenoic acid, 22:6n-3; EPA, eicosapentaenoic acid, 20:5n-3

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(Kharlamenko et al., 2008; Redmond et al., 2010), gastropods (Kharlamenko et al., 2011), crustaceans (Nerot et al., 2009) or suspension-feeding bivalves (Canuel et al., 1995; Napolitano et al., 1997; Silina and Zhukova, 2009). Some fatty acids cannot be synthesized *de novo* by consumers and, although most of them can be found in all food sources, few are specifically synthesized by primary producers. These fatty acids or groups of fatty acids, if assimilated, can therefore be used as trophic biomarkers to investigate the diet of benthic consumers (Kelly and Scheibling, 2012; Sargent et al., 1987). For instance, high contents of 18:4n-3 in consumers tissues suggest a diet dominated by dinoflagellates (Mansour et al., 1999; Silina and Zhukova, 2009; Alfaro et al., 2006), an enrichment in 16:1n-7 could reveal a diet mainly constituted of diatoms (Volkman et al., 1989; Meziane et al., 1997), and significant contents of branched (-iso and -anteiso) odd fatty acids or 18:1n-7 are often related to the consumption of benthic bacteria (Volkman et al., 1989).

Because of the commercial interest of Pectinids, numerous studies analyzed the dynamics of fatty acid composition in their tissues. Most studies focused on seasonal variations of energy allocation following the gametogenesis cycle (*e.g.* Caers et al., 2003; Palacios et al., 2005; Pazos et al., 1997), or on scallops nutrition requirements for improvements of hatchery efficiency (Caers et al., 1999; Soudant et al., 1996; Utting and Millican, 1998). Other studies assessed the relationship between the seasonal changes in the phytoplankton mixture and the temporal variations of fatty acid compositions of scallops in their natural environment (Napolitano and Ackman, 1993; Napolitano et al., 1997). However among these studies, very few evaluated their spatial variations. Temperature differences between different depth habitats were suggested to affect fatty acid composition of eggs and adductor muscle of *Placopecten magellanicus* (Napolitano et al., 1992), due to biochemical adjustments of cell membranes in response to the low temperatures prevailing at the greater depth. Silina and Zhukova (2007) showed an impact of bottom sediment types on feeding of *Patinopecten yessoensis*.

In a previous work, we investigated the spatial variability of Pectinids along an inshore–offshore gradient in the northern part of the Bay of Biscay, using stable isotopes (Nerot et al., 2012). We showed that the diet of *Pecten maximus* and *Aequipecten opercularis* could be partly explained by a decreasing gradient of benthic productivity with increasing depth, however, for deeper habitats, we were not able to accurately identify their trophic resource. Through the analysis of fatty acids in both muscle and digestive gland of *P. maximus*, the present study aims to complement the stable isotopes data in order to identify the food sources of the great scallop *P. maximus* along the same inshore–offshore gradient. Four sites were chosen as they represent different environmental conditions on the continental shelf of the Bay of Biscay (nearshore, coastal, mid-continental shelf, edge of continental slope). We determined the spatial variations in fatty acid compositions in two different organs: the digestive gland and the adductor muscle. These two organs respond differently to environmental changes, including food supply (Lorrain et al., 2002; Napolitano et al., 1997; Pazos et al., 2003). Fatty acid composition of the digestive gland is affected by dietary treatments (Pazos et al., 2003; Ventrella et al., 2013) and is expected to reflect the food ingested by the scallops over short periods as digestive gland incorporates a large number of food particles by phagocytosis (Napolitano and Ackman, 1993). In this organ, scallops also accumulate reserve lipids (*i.e.* neutral lipids) in this organ, which remains relatively unmodified by the diet (Giese, 1966). In contrast, muscles are expected to integrate dietary FA over longer time periods, but also to reflect the metabolic and physiological status of scallops because of selective retention. The composition of lipids in muscles is indeed dominated by phospholipids, whose composition is constantly modified according to the metabolic requirements of the animal.

2. Material and methods

2.1. Study area

Scallops were sampled for fatty acids analysis in May 2009, along an inshore–offshore gradient, from the Bay of Brest (4°28'W, 48°30'N) down to the limit of the continental shelf of the Northern Bay of Biscay (6°47'W, 47°49'N) (Fig. 1). The four sampling stations

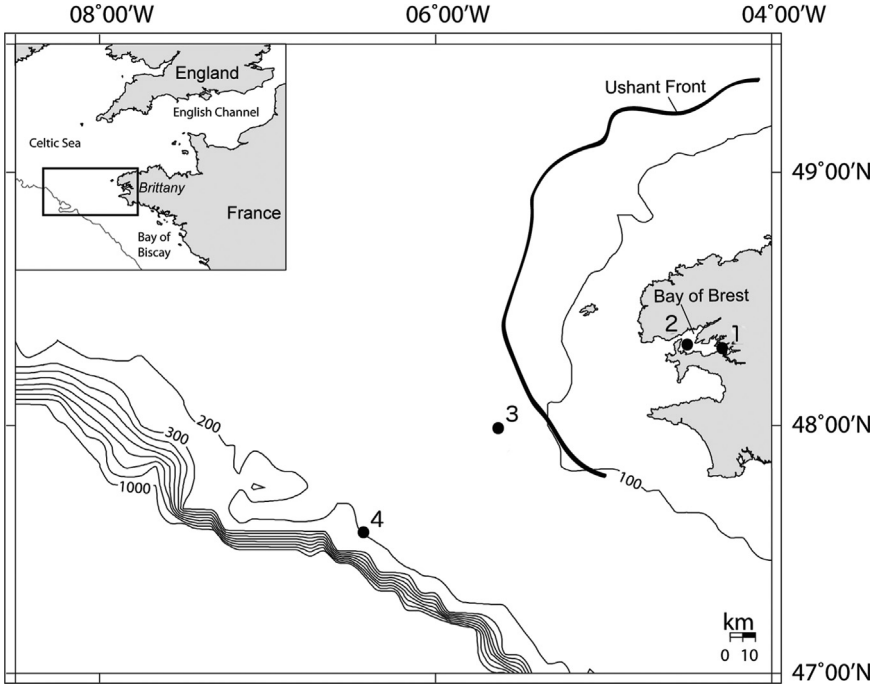

Fig. 1. Map of sampled stations in the northern part of the Bay of Biscay.

Table 1
Fatty acid composition (mean % TFA ± SD) of muscles and digestive glands of *Pecten maximus* for each station. Data represent all fatty acids at concentrations >1% TFA in at least one sample. tr = trace amounts < 0.1%.

Fatty acids	Digestive glands				Muscles			
	St. 2 (15 m)	St. 3 (24 m)	St. 9 (140 m)	St 11 (190 m)	St 2 (15 m)	St 3 (24 m)	St. 9 (140 m)	St 11 (190 m)
14:0	6.5 ± 0.1	6.4 ± 0.1	6.8 ± 1.0	5.3 ± 0.2	3.6 ± 0.1	4.5 ± 0.4	3.9 ± 0.3	3.2 ± 0.3
15:0	0.5 ± 0.0	0.4 ± 0.0	1.0 ± 0.2	0.7 ± 0.0	0.8 ± 0.0	1.0 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
16:0	16.9 ± 0.4	18.7 ± 0.2	23.5 ± 3.5	24.4 ± 1.2	21.4 ± 0.8	31.0 ± 1.5	24.7 ± 1.4	21.8 ± 1.0
17:0	0.7 ± 0.0	0.7 ± 0.1	1.9 ± 0.3	1.1 ± 0.1	1.2 ± 0.1	1.6 ± 0.1	2.4 ± 0.1	1.7 ± 0.1
18:0	4.0 ± 0.2	4.9 ± 0.3	1.2 ± 1.4	5.5 ± 0.6	7.8 ± 0.7	11.5 ± 1.2	10.4 ± 0.1	7.4 ± 0.5
20:0	0.3 ± 0.0	1.0 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.5 ± 0.3	1.4 ± 0.8	0.6 ± 0.5	0.2 ± 0.0
Total SFA	**28.8 ± 0.8**	**32.1 ± 0.6**	**40.6 ± 7.1**	**37.3 ± 2.3**	**35.3 ± 2.4**	**51.1 ± 3.1**	**43.5 ± 2.4**	**35.7 ± 1.7**
16:1n-7	7.9 ± 0.4	7.0 ± 0.2	4.5 ± 0.5	6.9 ± 0.5	2.0 ± 0.2	1.4 ± 0.1 ±	0.3	1.8 ± 0.2
18:1n-7	4.0 ± 0.1	3.8 ± 0.1	2.6 ± 0.3	3.7 ± 0.1	3.7 ± 0.3	3.0 ± 0.1	1.9 ± 0.3	2.2 ± 0.3
18:1n-9	1.9 ± 0.2	2.6 ± 0.1	5.4 ± 0.4	3.4 ± 0.2	1.5 ± 0.0	1.4 ± 0.1	1.6 ± 0.2	1.3 ± 0.1
20:1n-11	0.4 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.6 ± 0.3	0.4 ± 0.2	0.1 ± 0.0
20:1n-7	0.8 ± 0.0	0.9 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
20:1n-9	0.5 ± 0.1	0.8 ± 0.0	1.5 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	1.2 ± 0.1	2.1 ± 0.2	2.1 ± 0.2
Other MUFA[a]	**3.5 ± 0.5**	**2.8 ± 0.1**	**3.8 ± 1.3**	**2.8 ± 0.3**	**1.5 ± 0.2**	**1.2 ± 0.2**	**0.6 ± 0.1**	**0.5 ± 0.1**
Total MUFA	**18.9 ± 0.4**	**18.5 ± 0.0**	**18.9 ± 1.1**	**19.0 ± 0.6**	**10.4 ± 0.4**	**9.6 ± 0.7**	**8.5 ± 1.2**	**8.5 ± 0.3**
15:Oiso	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	tr	tr	tr	tr
17:Oanteiso	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
17:Oiso	0.4 ± 0.0	0.2 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	1.0 ± 0.1	1.0 ± 0.1	1.4 ± 0.1	1.1 ± 0.2
Total BFA	**0.6 ± 0.1**	**0.4 ± 0.0**	**1.2 ± 0.2**	**1.5 ± 0.1**	**1.2 ± 0.2**	**1.2 ± 0.1**	**1.6 ± 0.1**	**1.3 ± 0.2**
18:2n-6	1.3 ± 0.1	1.6 ± 0.0	1.8 ± 0.1	1.2 ± 0.1	1.0 ± 0.0	0.9 ± 0.1	0.7 ± 0.1	0.4 ± 0.1
18:3n-3	2.0 ± 0.1	1.9 ± 0.0	2.4 ± 0.3	1.0 ± 0.2	0.6 ± 0.1	0.4 ± 0.1	0.6 ± 0.0	0.5 ± 0.1
18:4n-3	7.9 ± 0.4	7.7 ± 0.2	7.0 ± 1.4	4.5 ± 0.4	2.8 ± 0.3	1.5 ± 0.2	2.5 ± 0.2	1.6 ± 0.3
20:4n-3	1.0 ± 0.0	1.2 ± 0.0	0.9 ± 0.3	0.7 ± 0.1	0.6 ± 0.2	0.8 ± 0.2	0.5 ± 0.2	0.5 ± 0.1
20:4n-6	1.4 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	1.0 ± 0.0	2.5 ± 0.1	1.7 ± 0.1	2.1 ± 0.2	2.1 ± 0.2
20:5n-3	21.6 ± 0.9	19.2 ± 0.6	9.6 ± 1.8	16.2 ± 0.9	18.2 ± 0.4	12.5 ± 0.8	10.9 ± 0.6	14.8 ± 0.4
22:4n-6	1.2 ± 0.0	1.5 ± 0.3	0.8 ± 0.2	0.9 ± 0.1	0.5 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
22:5n-3	0.6 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.5 ± 0.2	1.2 ± 0.0	0.7 ± 0.1	0.8 ± 0.1	1.0 ± 0.1
22:5n-6	0.3 ± 0.0	0.1 ± 0.0	0.7 ± 0.3	0.2 ± 0.0	0.8 ± 0.0	0.7 ± 0.2	1.1 ± 0.1	1.1 ± 0.1
22:6n-3	9.0 ± 0.2	9.1 ± 0.4	12.0 ± 2.8	10.5 ± 1.1	21.1 ± 1.9	15.2 ± 1.4	24.1 ± 2.5	28.8 ± 1.3
Other PUFA[b]	**3.0 ± 0.1**	**3.3 ± 0.1**	**2.1 ± 0.3**	**1.7 ± 0.1**	**3.4 ± 0.3**	**3.4 ± 0.3**	**2.8 ± 0.3**	**2.9 ± 0.2**
Total PUFA	**49.3 ± 1.0**	**47.3 ± 0.5**	**38.6 ± 8.3**	**40.5 ± 2.5**	**52.8 ± 0.3**	**37.9 ± 2.6**	**46.2 ± 3.5**	**54.1 ± 2.0**
16:1/16:0	0.47	0.37	0.19	0.28	0.09	0.05	0.06	0.08
DHA/EPA	0.42	0.47	1.25	0.58	1.16	1.21	2.20	1.95

[a] MUFA with amount <1% (14:1, 14:1n-5, 16:1n-5, 17:1).
[b] PUFA with amount <1% (16:2n-4, 18:2n-4, 18:3n-6, 20:2, 20:2n-6, 21:5n-3, 22:4n-3. 22:4n-9trans).

were chosen along a gradient of increasing depth and decreasing terrestrial influence, from very nearshore (stations 1 and 2) to the limit between the continental shelf and the continental slope. Stations 1 (15 m depth) and 2 (24 m depth) are both located in the Bay of Brest, a shallow coastal bay linked to the Atlantic ocean by a narrow strait about 1.8 km wide. Station 1 is located near the Aulne river mouth, with seasonal fluctuations of river and terrestrial inputs (Nerot et al., 2012). Station 2 is located at the entrance of the bay, characterized by strong and daily water exchanges with the Iroise Sea. High frequency environmental data are available for this sampling station (SOMLIT, http://somlit.epoc.u-bordeaux1.fr/fr/) and it is a well-studied site for scallop ecophysiology (Lorrain et al., 2002; Paulet et al., 2006). Stations 3 (140 m depth) and 4 (190 m depth) are both located offshore, but in two distinct water masses. The water column at station 3 is stratified, with the presence of a deep cold water mass present all over the year with low temperature variations. Station 4, at the edge of the continental shelf, is characterized by a vertical mixing of the water column (Lazure et al., 2009).

2.2. Sample collection and preparation

Individual great scallops *Pecten maximus* were collected at each station by dredging (scallop dredge, 50 mm opening mesh) in May 2009. Because *P. maximus* typically exhibits low densities at deep sites (*i.e.* >100 m depth), only three to four individuals were analyzed for each station. However, FA composition of benthic consumers typically experience low variability (Meziane and Tsuchiya, 2000; Nerot et al., 2009) and individual FA proportions are generally similar between replicates, therefore limiting the potential bias induced by reduced samples replication. Adductor muscles and digestive glands of scallops were quickly dissected and stored frozen (−80 °C) before lipid extraction of a weighted aliquot (~200 mg for digestive gland and ~500 mg for muscle). Lipids were extracted from tissues in a glass Dounce tissue grinder with about a 30-fold (digestive gland) and a 16-fold (muscle) volume of solvent mixture ($CHCl_3$/MeOH 2:1, v:v), following the method described by Folch et al. (1957). Extracted lipids (in chloroform) were dried under nitrogen and then saponified under reflux (2 h, 100 °C) with a 2 mol dm^{-3} NaOH solution in methanol and distilled water (2:1, v:v). Saponification and methylation were carried out according to Meziane and Tsuchiya (2002) in order to gather and methylate all FAs. FA methyl esters (FAMEs) were then purified by high performance thin-layer chromatography technique (HPTLC) using plates coated with silicagel. The developing solvent was a mixture of hexane/diethylether/acetic acid (8:2:0.1). Bands containing FAMEs were scraped and collected in a mixture of chloroform/methanol (2:1, v:v) at 40 °C for 60 min. FAMEs were then isolated in the same solution until analysis by gas chromatography.

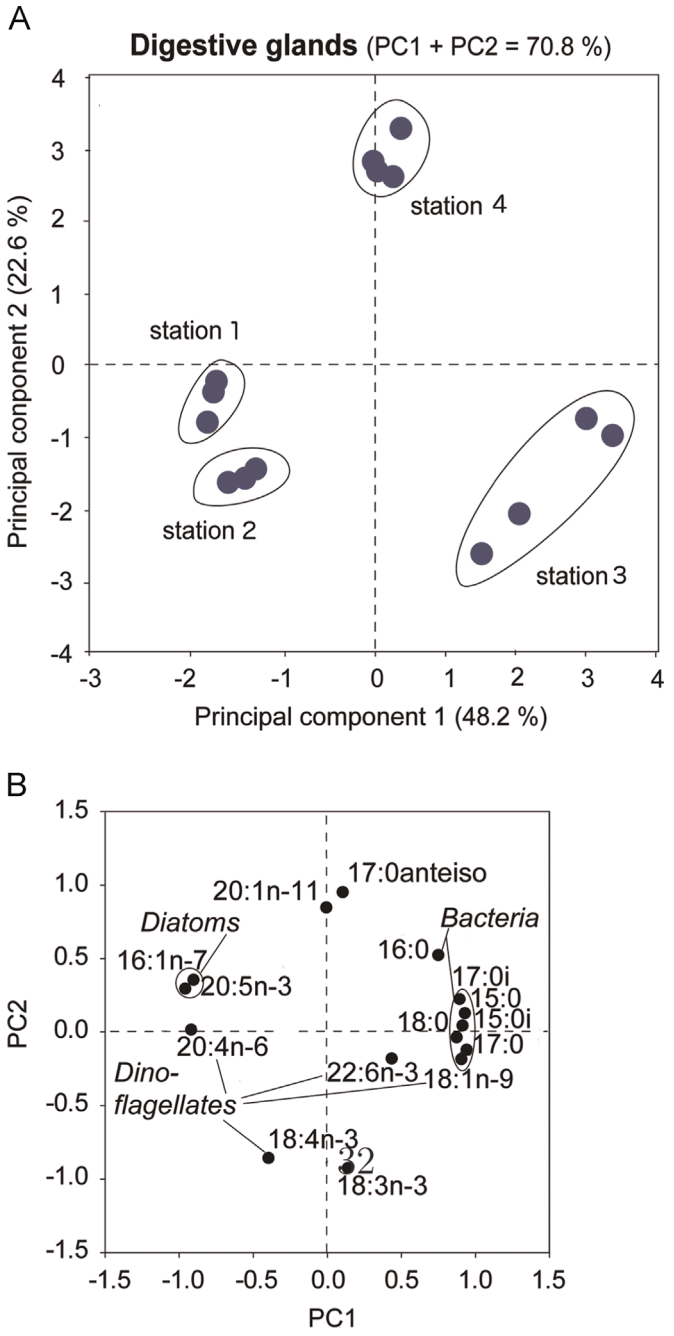

Fig. 2. Principal component analysis (PCA) of transformed proportional data of *Pecten maximus* digestive glands collected at the four sites along the depth transect: (A) PCA scores plot to show separation of individuals (stations 1, 2: $n = 3$. stations 3, 4: $n = 4$) among sites. Circles indicate sites that are significantly different as determined by one-way ANOVA performed on the scores; (B) PCA loadings (>0.5 for at least one component) to show individual fatty acids that contribute to the separation among sites.

2.3. Fatty acids analysis

The fatty acids were separated and quantified by gas chromatography (Varian CP3800) equipped with a polar Supelco – OMEGAWAX 320 capillary column (30 m × 0.32 mm; 0.25 µm film thickness) and a flame ionization detector; with hydrogen as carrier gas. After injection at 60 °C, the oven temperature was raised to 150 °C at a rate of 40 °C min^{-1}, then to 240 °C at 3 °C min^{-1}, and finally held constant for 30 min. Flame ionization was held at 240 °C. Identification of FAMEs was performed by comparing their retention times with those of known standards (37 components FAME Mix, Supelco) and, when necessary, were confirmed by mass spectrometry (Varian Saturn CP2200). Contribution of each individual fatty acids in a tissue is given in relative value of total fatty acids (% of TFA), based on the area of the respective peak in the GC-FID spectra.

2.4. Data analysis

All fatty acids proportional data were arcsin transformed for standardization and to improve normality. Separate principal component analyses (PCA) were performed on proportional fatty acids' profiles for muscle and digestive gland samples to reveal spatial variability among the locations in these organs. In FA analysis, the use of PCA allows description of relationships among variables and also identification of specific FA that represent most of the variance in the data set (Budge et al., 2006). Analyses of variance (ANOVA) were processed on PCA scores to reveal the most influent FA in the PCA analysis. ANOVA were also processed on individual fatty acids with post hoc Fisher test to highlight differences between the four locations. All tests are considered significant when p-value <0.05.

3. Results

3.1. Pecten maximus digestive glands

A total of 25 fatty acids were detected in proportion >1% of TFA in at least one digestive gland sample (Table 1). Dominant fatty acids were 20:5n-3 (9.6–21.6%), 16:0 (16.9–24.4%), and 22:6n-3 (9.0–12.0%). There were also elevated proportions of 16:1n-7 (4.5–7.9%) and 18:4n-3 (4.5–7.9%). The plot of PCA scores revealed a strong homogeneity between samples from the same location and differences among locations (Fig. 2). The two components explained 70.8% of the total variance. The PC1 explained 48.2% and discriminated stations 1 and 2 (clustered on the left side of the plot) from station 3 (clustered on right side of the plot), with station 4 being intermediate. The main fatty acids counting for variation in PC1 were the odd saturated 17:0 and 15:0, the branched 15:0iso and 17:0iso, together with the 16:1n-7, 18:1n-9, 20:4n-6 and 20:5n-3 (sum of contributions to PC1 = 35%). The PC2 explained 22.6% of the variance. Variation along PC2 was influenced at 39% by the 17:0iso, 18:3n-3, 20:1n-11 and 18:4n-3.

Analysis of individual fatty acids or FA ratios in digestive glands revealed specific differences among stations (Fig. 3). Digestive glands from stations 1 and 2 showed similar fatty acid compositions, except for the low but significant differences of 16:1n-7 and 18:1n-9 fatty acids (Fig. 3). In comparison to other stations, digestive glands from station 3 had lower values of EPA (20:5n-3) and 16:1n-7, while they exhibit higher proportion of branched fatty acids (BFA), 15:0, 17:0 and 18:1n-9. Fatty acid composition of digestive glands from station 4 was globally similar to that of station 2 but shows higher values for BFA, 15:0, 17:0, 18:4n-3 and 18:1n-9. The DHA/EPA ratio was <1 at stations 1, 2 and 4 while it was >1 at station 3 (1.25).

3.2. Pecten maximus adductor muscles

In muscle tissues, 24 fatty acids were detected with proportion >1% in at least one sample (Table 1). Dominant fatty acids in muscle were 16:0 (21.4–31.0%), 22:6n-3 (15.2–28.8%) and 20:5n-3 (10.9–18.2%). There were also high proportions of the saturated 18:0 (7.4–11.5%).

The first components of the PCA explained 63.1% of the variance in the data set of the muscle fatty acids composition (Fig. 4). Spatial repartition of individual samples revealed homogeneity

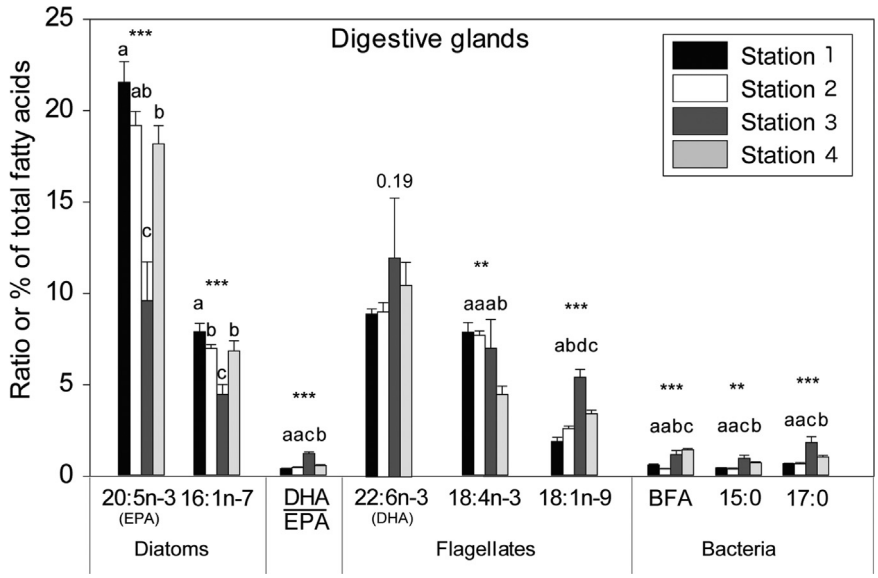

Fig. 3. Composition of fatty acids selected as source indicators in digestive glands of *Pecten maximus* (ratio or % TFA ± SD, stations 1, 2: $n = 3$. stations 3, 4: $n = 4$) in the four sampling stations. The selected fatty acids are those used as markers for consumption of diatoms, flagellates and bacteria. Significant tests denoted with asterisks ($^{*}p < 0.05$;** $p < 0.01$;*** $p < 0.0001$). Mean values with the same letter are not significantly different (one-way ANOVAs with Fisher post-hoc $\alpha = 0.05$). Alphabetical order reflects values ranking.

within samples from a same station and variability among stations, which resulted in a spatial separation of the four stations in the PCA plot. The PC1 explained 33.4% of variance and clustered stations 3 and 4 on the left side of the scores plot and stations 1 and 2 on the right side. The fatty acids with the highest contribution to PC1 axis were the PUFA 18:2n-4, 18:3n-6, 22:5n-3 and 22:6n-3, together with the MUFA 18:1n-7 (sum of contributions = 34.8%) (Fig. 4B). The PC2 explained 29.7% and separated stations 1 and 4 on top and stations 3 and 2 on bottom. Six fatty acids cumulated 40.7% of variation, 20:5n-3, 22:5n-3, 21:0, 18:0, 20:4n-6 and 16:1n-7.

Muscle samples from station 1 showed higher proportions of EPA (20:5n-3) than in other stations (p-values <0.0001) (Table 1, Fig. 5). The DHA (22:6n-3) content in muscles was the highest in deep water scallops, and the lowest content was found in scallops from station 2. The DHA/EPA ratio was always higher than 1 and was higher at stations 3 and 4 than at stations 1 and 2 ($p = 0.0098$). Scallops from stations 1 and 4 showed similar contents of 16:0, lower than at station 2. This pattern of the 16:0 and DHA influenced the total SFA and PUFA contents: PUFA dominated at stations 1, 3 and 4, while SFA were the major group at station 2. Total MUFA were similar at all stations.

Apart from DHA and EPA, proportions of fatty acids used as trophic markers were lower than 5% in muscles (Fig. 5). Scallops from stations 1 and 2 showed different levels for every trophic marker FA except 18:1n-9 and total branched FA. In deep stations 3 and 4, scallops muscle showed different FA proportions, except for the odd saturated 15:0.

4. Discussion

4.1. Fatty acids information from digestive glands

The digestive glands of *P. maximus* exhibited distinct FA composition with regard to location. Among scallops FA that contributed the most to the total variability of the dataset, some of them were commonly considered as trophic biomarkers, suggesting that variability in FA composition along this depth gradient is strongly related to a variability in their diet (Kelly and Scheibling, 2012). The scallops from the two shallow areas in the Bay of Brest were characterized by higher contents of 16:1n-7 and 20:5n-3 that are usually abundant in diatoms (Dalsgaard et al., 2003). 22:6n-3 and 18:4n-3, often considered as indicative of dinoflagellates contribution, were less abundant than 20:5n-3, but present at similar proportions as 16:1n-7 in scallops from these two stations (around 9% of TFA). This may indicate that although nearshore scallops predominantly rely on diatoms for food, dinoflagellates also contribute to their diet. Blooms of both diatoms and dinoflagellates in the Bay of Brest in 2009 have been documented (SOMLIT data; http://somlit.epoc.u-bordeaux1.fr). Moreover, Ragueneau et al. (1994) and delAmo et al. (1997) found that phytoplankton blooms are largely dominated by diatoms, dinoflagellates being only secondary components of phytoplankton productivity. This dominance could explain the abundance of diatoms over dinoflagellates FA markers in digestive glands of scallops.

Although our results indicate diatoms as the dominant food source for scallops, still FAs cannot tell whether these consumers rely on planktonic or on benthic microalgae (Dalsgaard et al., 2003). Previous studies have suggested that benthic diatoms should represent a major proportion of *P. maximus* diet (Grall et al., 2006; Lorrain et al., 2002). Moreover, these diatoms are known to represent a significant part of primary production in this coastal ecosystem (Longphuirt et al., 2006). It is therefore very likely that benthic diatoms significantly contribute to the diet of scallops at coastal sites. Overall, the relative similarity in the FA composition of scallops from the two shallow water stations of the present study suggests that their diet are similar, and are clearly different from scallops sampled at deeper sites. Parrish et al. (2009) used the DHA/EPA ratio to measure the relative dominance of diatoms (ratio < 1) or dinoflagellates (ratio > 1) biomasses. At station 3, one could interpret the high DHA/EPA ratio (1.25) as a dominance of dinoflagellates in the diet of scallops, which is confirmed by significant amounts of 18:4n-3. The water column at station 3 is stratified year round due to a permanent thermocline (Lazure et al., 2009; Puillat et al., 2004). Such a stratified water column with no excessive turbidity has been reported to represent favorable conditions for growth of dinoflagellates (Margalef, 1978). However, the low PUFA content found in digestive glands of scallops at this station (37.7% of

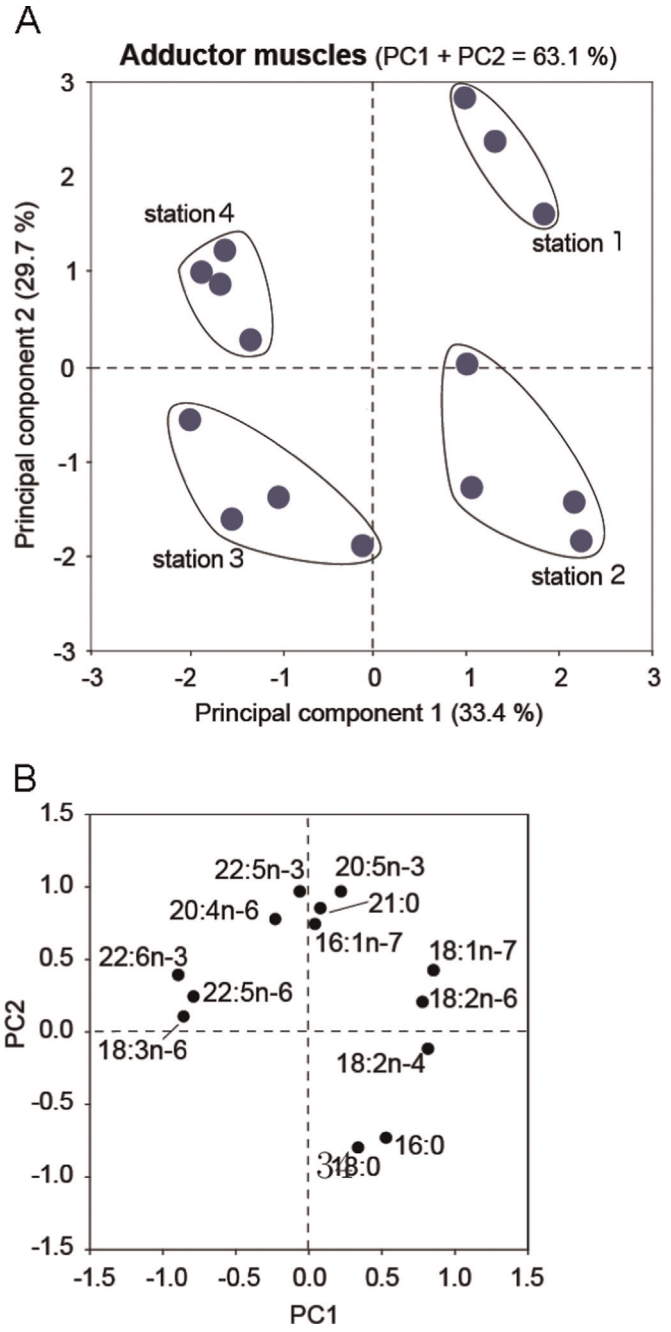

Fig. 4. Principal component analysis (PCA) of transformed proportional data of *Pecten maximus* adductor muscles collected at the four sites along the depth transect: (A) PCA scores plot to show separation of individuals ($n = 3$ at station 1. $n = 4$ at stations 2, 3, 4) among sites. Circles indicate sites that are significantly different as determined by one-way ANOVA performed on the scores; (B) PCA loadings (>0.5 for at least one component) to show individual fatty acids that contribute to the separation among sites.

TFA), as well as the relative importance of bacterial markers (odd and branched FA), does not support the hypothesis of a food chain mainly based on microalgae, that are one of the major sources of PUFA in the marine environment (Dalsgaard et al., 2003). Scallops from this station live below the euphotic zone (~30 m in Western Brittany), where there should be no local photosynthetic activity close to the seafloor and particles sinking is likely limited by the presence of the permanent stratification (Lazure et al., 2009; Puillat et al., 2004). Another hypothesis explaining the higher relative importance of DHA for this station is the higher contribution of microheterotrophs to the diet of *P. maximus*. Although FA composition data for marine flagellates and ciliates are scarce, few studies focusing on these groups found that they can represent a significant source of PUFA in a marine environment (Zhukova and Kharlamenko, 1999). For instance, the flagellate *Bodo* sp. was found to have a high ability of DHA biosynthesis. Thus in station 3, ingestion of this flagellate by the scallops could explain the presence of this PUFA in their digestive glands (Balzano et al., 2011). The FA 18:1n-9, also found in higher abundance in the scallops of this station, was also abundant in two common flagellates from the Bay of Biscay, *Bodo* sp. and *Euplotes* sp., and has been reported to be especially abundant in detrital particles (Balzano et al., 2011). The hypothesis of flagellates contribution at this station is also supported by the high bacterial markers abundance, as well as by lower total PUFA abundance than at other stations. Compared to microalgae-based food chains, bacterial loop-based food chains are characterized by lower PUFAs abundances (Oliver and Colwell, 1973). In the microbial loop, intermediate consumers (*i.e.* flagellates and ciliates) have shown some capacity to synthesize *de novo* PUFAs (Zhukova and Kharlamenko, 1999), but the PUFA content of consumers relying on the microbial loop is generally lower than for consumers feeding on microalgae (Dalsgaard et al., 2003). According to this hypothesis, both DHA and EPA of microalgal origin would be less available for scallops inhabiting this area than for individuals found in shallower areas, but DHA inputs would be compensated by the abundance of microheterotrophs feeding on bacteria. Consequently, the high DHA/EPA ratio found for this station would not indicate a predominance of dinoflagellates over diatoms, which suggests that this FA indicator might not be suitable for deep aphotic ecosystems. Besides, it has been reported that in case of dietary deficiency, which is likely the case in deep environment with no local primary production or virtually no vertical fluxes of particles, *P. maximus* is able to maintain relatively high content of DHA, which is used for metabolic processes, at the expense of EPA (Soudant et al., 1996).

Station 4 (190 m) is located at the edge of the continental shelf, and is the maximal depth limit of *Pecten maximus* (Brand et al., 2006). From a hydrological point of view, station 4 differs from station 3 through the absence of stratification (Lazure et al., 2009; Puillat et al., 2004), which likely enhances water mixing and vertical transfer of microalgal productivity to the benthic zone (Bergeron et al., 2010; Duchemin et al., 2008). Composition of fatty acids in scallops sampled at this station were unusual, as their digestive gland strongly differs from scallops at station 3. In particular, EPA and 16:1n-7 were more abundant than in station 3 and close to the contents found at the shallow water scallops. As these two FAs are commonly considered as biomarkers of diatoms, this firmly suggests that these microalgae support the growth of scallops at the limit of the continental shelf. Stronger water mixing compared to station 3 would therefore result in an increased availability of phytoplankton to scallops. At the vicinity of the continental slope, inputs of cooler and nutrient-rich waters to the surface (Bergeron et al., 2010) are responsible of favorable growth conditions for diatoms (Joint et al., 2001). In contrast, the amount of bacterial markers was quite similar to what was found at station 3, whereas relative abundance of DHA and 18:1n-9 were intermediate between shallow areas and station 3, suggesting that the microbial loop contribution in the diet of these continental shelf scallops is not negligible.

4.2. Fatty acids information from muscles

In the current study, the FA compositions of muscles were difficult to interpret from a trophic point of view because of the low amounts of trophic marker FAs, which indicate strong regulations of muscle FA composition compared to dietary input. Indeed, fatty acids in muscles of Pectinids are not considered to only

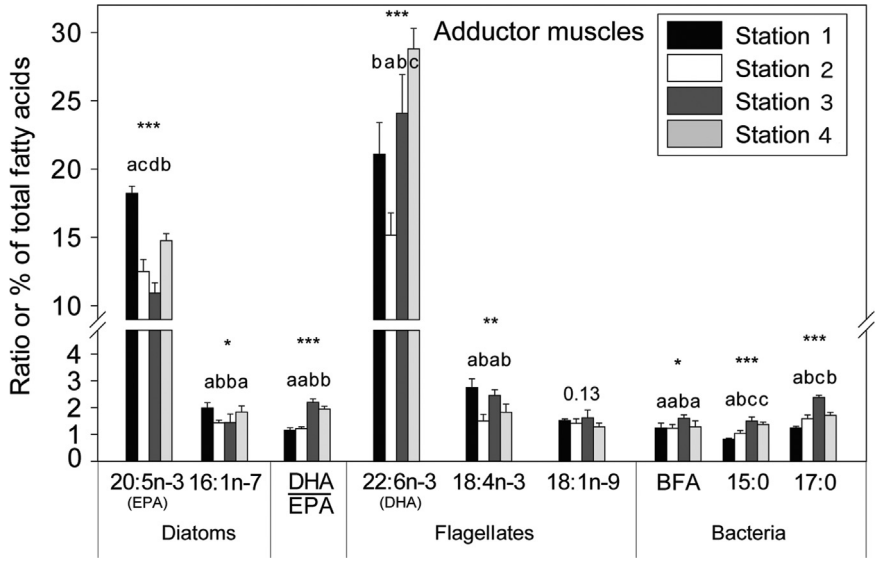

Fig. 5. Composition of fatty acids selected as source indicators in adductor muscles of *P. maximus* (ratio or % TFA ± SD, $n = 3$ at station 1. $n = 4$ at stations 2, 3, 4) in the four sampling stations. The selected fatty acids are those used as markers for consumption of diatoms, flagellates and bacteria. Significant tests denoted with asterisks ($*p < 0.05$;$**$ $p < 0.01$;$***$ $p < 0.0001$). Mean values with the same letter are not significantly different (one-way ANOVAs with Fisher post-hoc tests; $\alpha = 0.05$). Alphabetical order reflects values ranking.

reflect recently ingested food sources, but are also determined by endogenous processes such as energy transfer to gonad for gametogenesis (Napolitano and Ackman, 1993; Pazos et al., 1997), and environmental conditions such as temperature. These parameters lead to structural and functional adjustments of cellular membranes for physiological adaptation to their physical environment (*e.g.* Silina and Zhukova, 2007; Telahigue et al., 2010).

Scallops from stations 1 and 2 exhibited different relative proportions of major FA in muscles (Table 1). PUFAs and particularly 22:6n-3, which are considered to be physiologically needed for Pectinids, were the major muscle FAs accounting for this difference. A selective mobilization of 22:6n-3 from the muscle tissues to the gonad for maturation has been described for *P. maximus* (Soudant et al., 1996) and *Nodipecten subnodosus* (Palacios et al., 2005). In the Bay of Brest, energy mobilization for gonad maturation and gametogenesis for *P. maximus* occurs from November to April (Lorrain et al., 2002; Saout et al., 1999). In the present study, scallops were sampled in early spring 2009, when temperature and food supply increase, leading to initial maturation and spawning in *P. maximus* (Devauchelle and Mingant, 1991; Saout et al., 1999). The differences of FA composition observed in muscle from stations 1 and 2, and particularly of 22:6n-3 contents, suggest that scallops may have different gametogenesis and reproduction dynamics at these two stations in the Bay of Brest.

In deep water stations 3 and 4, muscles of scallops exhibited lower amounts of SFA and higher amounts of PUFA than in the shallow water stations. Shallow and deep scallop muscles were significantly separated by the PCA analysis (Fig. 4), suggesting an environmental impact and a physiological importance of their FA composition. Water depth and latitude both determine the boundaries of distribution ranges for a given species (Osovitz and Hofmann, 2007). Peck et al. (2009) showed that organisms sampled at their latitudinal limits exhibit a reduced metabolism, which should also apply for the depth limits. While the water temperature in the Bay of Brest is highly variable year round (10–20 °C, Chauvaud et al., 2000), water temperature on the continental shelf is stable and in the cold range (~11°C year round, Pingree et al., 1982). An important feature of temperature adaptation is the biochemical adjustment of membrane fluidity and functionality (Hazel and Williams, 1990), through strong regulation of essential FA in cellular membranes such as EPA and DHA (Crockett, 2008). By comparing individuals of *Placopecten magellanicus* from two depth different sites (10 and 31 m), Napolitano et al. (1992) concluded that the differences in their fatty acid compositions were related to the site-specific temperatures. Furthermore, daylight does not reach the sea floor at the deep water stations, resulting in the absence of light cycles for these scallops. The metabolic processes that usually follow temperature and diurnal and seasonal light cycles such as reproduction and growth may therefore be strongly reduced for deep scallops. We also suggest that, given the water depths reached in the current study (190 m), the FA composition of membrane phospholipids in muscles of scallops may represent a homeoviscous adaptation to important hydrostatic pressure (Cossins and Macdonald, 1986). Impact of environmental conditions such as temperature variability, light cycles and pressure in deep waters on metabolic functioning of scallops were similarly raised through surprising stable isotopes results at stations 3 and 4 (Nerot et al., 2012). Further work on the impact of these factors, particularly pressure, on the FA composition of bivalve tissues should help to clarify this question.

5. Conclusion

This study used fatty acids to investigate the diet of the great scallop *Pecten maximus* over its depth range in the Bay of Biscay. Fatty acid compositions in scallop tissues proved to be complementary to a former study of spatial variations in carbon and nitrogen stable isotopes that aimed to identify food sources of scallops along the depth gradient, especially for species inhabiting deeper waters. Considering the FA composition of digestive glands, a strong difference in the diet of scallops was found between shallow and deep water habitats, mostly driven by phytoplankton dynamics, with a non-negligible contribution of microheterotrophs and microbial loop at the deeper stations. This site specific FA composition of digestive glands showed the role of hydrological structures of the continental shelf on the prevalent food sources that contributed to the diet of *P. maximus*. On the other hand, site specific FA composition of muscles highlighted the influence of the contrasting environmental factors on metabolic and physiological status of scallops along this depth range of spatial distribution.

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