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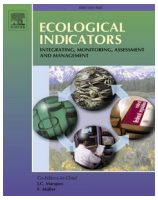

Original Articles

Prevalence of phylogenetic over environmental drivers on the fatty acid profiles of the adductor muscle of marine bivalves and its relevance for traceability

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ABSTRACT

Marine bivalves are among the most traded seafood worldwide. Along with their economic value, they are also an excellent source of proteins, vitamins, minerals, omega-3 and omega-6 fatty acids (FA). The FA profiles of the adductor muscle (AM) of bivalves can be successfully used to trace their geographic origin. This approach is paramount for traceability, as it allows to expose fraudulent practices associated with the capture of bivalves from unsuitable areas for human consumption that may put food safety at risk. However, it is yet to be determined whether phylogenetic or environmental drivers prevail on the shaping of FA signatures when comparing multiple bivalve species originating from the same location or distinct geographic origins. In this study, the FA profiles of the AM of six commercially important bivalve species (*Cerastoderme edule, Crassostrea gigas, Mytilus galloprovincialis, Ruditapes philippinarum, Scrobicularia plana and Solen marginatus*) from Ria de Aveiro (Northwest Coast of Portugal) were compared. Furthermore, the FA profiles of the AM of *C. edule* and *R. phillipinarum* originating from Ria de Aveiro and the Tagus estuary (~250 Km apart) were also compared to identify if phylogenetic drivers prevailed over environmental ones. The FA profiles of the AM of the six bivalve species sampled in Ria de Aveiro differed significantly among themselves, displaying higher levels of similarity between species belonging to the same infraclass. The comparison of species from the same ecosystem and specimens from different ecosystems evidenced that phylogenetic drivers prevail over environmental ones on the shaping of FA profiles of the AM. These findings are important for food safety issues, as they revealed that the FA profile of the AM of a given bivalve species cannot be used as a reliable proxy for another one when aiming to trace geographic origin. The transfer of this technology to bivalve's production ensures product's safety, promotion and differentiation, as well as a tool against fraud.

1. Introduction

Bivalves are among the most economically valuable marine living resources (Wijsman et al., 2019). The global production of these organisms has been increasing over the years, from one million tons in 1950 to more than 17 million tons in 2018 (FAO, 2020). Bivalves are important for human diet, mostly due to their high nutritional value, low level of calories and high content in proteins, vitamins, minerals, omega-3 and omega-6 fatty acids (FA) (Wright et al., 2018).

The FA profiles of marine bivalves are influenced by intrinsic (e.g. sex, maturity and phylogeny) and external factors (e.g. diet, salinity and temperature) (Calado and Leal, 2015; Dalsgaard et al., 2003; Fokina et al., 2015; Kraffe et al., 2008; Olsen et al., 2009; Zhukova, 2019). Bivalves colonize a multitude of habitats that are shaped by contrasting environmental drivers. For instance, oysters and mussels are commonly present in intertidal areas attached to hard-substrates, while clams and cockles live borrowed in sediment of variable grain-size. These contrasting groups of bivalves evolved metabolic mechanisms that enable them to adapt and cope with variable ranges of environmental shifts, such as those of salinity or water temperature (Chapelle, 1987). Indeed,

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Kraffe et al. (2008) described a close relationship between the FA profiles of mitochondrial cardiolipins of bivalves and their phylogeny, while Makhutova et al. (2011), in a study comprising different phyla of benthic macrofauna, confirmed that along with phylogeny, trophic strategies also influenced the FA profiles of these organisms. High levels of the FA 16:0 (palmitic acid, PA), 16:1*n*-7 (palmitoleic acid) and 20:5*n*-3 (eicosapentaenoic acid, EPA) in bivalve tissues reveal a diet rich in diatoms, while the prevalence of 18:4*n*-3 (stearidonic acid) and 22:6*n*-3 (docosahexaenoic acid, DHA) is associated with the ingestion of dinoflagellates; the presence of 18:2*n*-6 (linoleic acid, LA) and 18:3*n*-3 (linolenic acid, ALA) commonly results from the consumption of green microalgae, while odd chain FA (15:0, pentadecanoic acid; and 17:0, heptadecanoic acid) and 18:1*n*-7 (vaccenic acid) build-up due to the feeding on detritus and/or bacteria (Calado and Leal, 2015; Dalsgaard et al., 2003; Ezgeta-Balić et al., 2012; Nerot et al., 2015). Furthermore, it is well established that environmental factors can change the fatty acid composition of cell membranes in marine organisms (Dalsgaard et al., 2003). It has been reported that high salinity fluctuations and low water temperatures influence the fluidity and structure of cell membranes as a consequence of their FA composition, with membrane fluidity being secured by a replacement of saturated FA (SFA) by polyunsaturated FA (PUFA; Fokina et al., 2015; Nemova et al., 2013). These features have allowed to successfully use the FA profiles of different bivalve species as natural barcodes for diverse purposes, such as to trace their producing areas (Mamede et al., 2020; Ricardo et al., 2017a; Ricardo et al., 2015), feeding habitats (Freites et al., 2002), stress induced by contaminants (Filimonova et al., 2016 and references therein; Gonçalves et al., 2016) or diet composition (Xu and Yang, 2007). The use of FA profiles of the adductor muscle (AM) of bivalves as natural fingerprints of the environmental drivers they experience during their lifetime is particularly interesting. The high content of polar lipids present on the tissues of the AM is not as influenced as other bivalve tissues by the short-term turnover of FA related to dietary shifts, hence being more prone to reflect mid/long-term variations caused by abiotic conditions (Grahl-Nielsen et al., 2010; Leal et al., 2015; Paulet et al., 2006).

Despite their high nutritional value, the consumption of bivalves can present a risk to human health if these originate from polluted areas (Rippey, 1994; Stabili et al., 2013). The suspension feeding nature of bivalves favors the bioaccumulation in their tissues of toxins, pathogenic bacteria, viruses, metals and metalloids present in the water and sediments where they grow (Rippey, 1994; Stabili et al., 2013; Velez et al., 2015). Knowing the geographic origin of bivalves is therefore of utmost importance to safeguard the health of human consumers. For this purpose, several European pieces of legislation have already been published, either classifying production areas of seafood according to the levels of *Escherichia coli* recorded in the flesh and intra-valvular liquid of live bivalve specimens (quantified through a 5-tubes 3-dilution most probable number (MPN); Regulation (EC) No 853/2004, No 854/2004, No 2073/2005 and No 1021/2008)) (EC, 2008; EC, 2005; EC, 2004a; EC, 2004b), promoting the certification of origin of seafood through regulation framing organic aquaculture animal and seaweed production (Regulation (EC) No 710/2009) (EC, 2009a) and labelling regulations (Regulation (European Commission (EC)) No 104/2000 and No 1224/2009; Regulation (EU) No 404/2011 and No 1379/2013) (EC, 2009b; EC, 2000; EU, 2013; EU, 2011). The overall success of these legal measures stands, among other things, on the development of reliable traceability tools to confirm claims on the geographic origin of bivalves being traded for human consumption (Leal et al., 2015). These tools are commonly based on reference models that require a significant sampling effort due to the need to collect specimens from multiple harvesting locations (see Mamede et al., 2020; Ricardo et al., 2017a). Moreover, all specimens being screened must be collected within a short time window to prevent bias promoted by temporal (either seasonal or interannual) variability (Ezgeta-Balić et al., 2012; Ricardo et al., 2017b). To overcome these issues, it would be important to understand if a reference model assembled to determine the geographic origin for a given bivalve species could also be reliably used for other bivalve species occurring in the same sampling location.

The present study aimed to determine if the FA profiles of the AM of different bivalve species with commercial relevance display significant differences when these originate from the same sampling location. Additionally, it is also evaluated if phylogenetic-based drivers are more powerful shaping FA fingerprints of the AM of bivalves than ecosystem-specific environmental drivers experienced by these organisms.

2. Material and methods

2.1. Bivalve collection

In order to evaluate how similar could be the FA profiles of the AM of different bivalve species with commercial relevance originating from the same ecosystem, four specimens (commercial size) of six commercially important bivalve species were sampled over the Summer of 2014 on four adjacent transects in an oyster farm located in the Mira Channel of Ria de Aveiro (RAv), Portugal (40°35′58.30″N, 8°44′47.80″W, Fig. 1). The species sampled were *Cerastoderme edule* (Common cockle), *Crassostrea gigas* (Pacific cupped oyster), *Mytilus galloprovincialis* (Mediterranean mussel), *Ruditapes philippinarum* (Manila clam), *Scrobicularia plana* (Peppery furrow) and *Solen marginatus* (European razor clam) (1 ecosystem × 6 species × 4 transects × 4 samples = 96 samples; Table S1). The four transects (T1 to T4) were oriented perpendicular to the channel margins (Fig. 1).

In order to evaluate which driver (ecosystem-specific environmental drivers or phylogenetic-based programming) played a stronger role in the shaping of the FA profiles of the AM, specimens of *C. edule* and *R. philippinarum* collected in RAv were compared with conspecifics collected from the Tagus estuary (38°39′27.44″N, 9°6′35.95″W) also in the Summer of 2014 (TE; Fig. 1). This analysis comprised ten specimens of *C. edule* and *R. philippinarum* collected from RAv (randomly selected from previous analysis) and TE (2 ecosystem × 2 species × 10 samples = 40 samples). All samples were collected by hand-ranking, stored in aseptic plastic bags, and kept refrigerated until arrival to the laboratory. The taxonomic identity of all sampled specimens was confirmed, with the AM of each bivalve being dissected in the same day of sampling and stored at −80 °C until FA analysis.

2.2. Fatty acids analysis

The AM of all bivalves collected were weighed and homogenized, with total lipids being extracted with methanol/chloroform (2:1 v/v), according to Bligh and Dyer (1959) method. The lipids were then transesterified using C21:0 internal standard (1.25 μg.mL^{-1} in n-hexane) following the procedure described by Aued-Pimentel et al. (2004) to obtain FA methyl esters (FAME). The resulting FAME were identified using a gas chromatography coupled with mass spectrometry (GC-MS) on an Agilent Technologies 6890N Network (Santa Clara, CA) equipped with a DB-FFAP column (30 m × 0.32 mm internal × 0.25 μm) (J&W Scientific, Folsom, CA). The GC equipment was connected to an Agilent 5973 Network Mass Selective Detector operating with an electron impact mode at 70 eV and scanning the range *m/z* 50–550. The following temperature ramp was employed: 80 °C to 220 °C at 14.4 °C min^{-1}, subsequently at 10 °C min^{-1} to 240 °C, and then at 5 °C min^{-1} to 250 °C. Helium was used as the carrier gas at a flow rate of 0.5 mL min^{-1}. The injector and detector temperatures were programmed to be held at 220 °C and 280 °C, respectively. Chromatogram peaks were integrated and identified by comparison of retention times to a mixture of standards (C6-C24, Supelco 37 component Fame Mix) and analysis of MS spectra compared with MS spectra of standards. The results were expressed in relative abundance of total pool of FA as average values ± standard deviation.

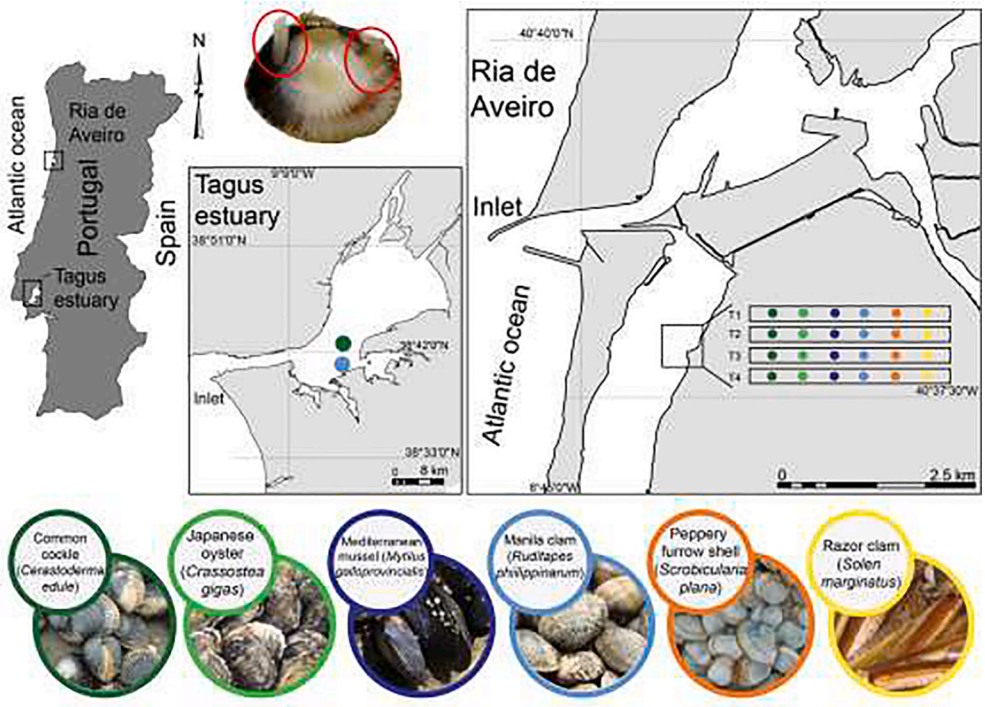

Fig. 1. Sampling locations of six bivalve species (*Cerastoderme edule*, *Crassostrea gigas*, *Mytilus galloprovincialis*, *Scrobicularia plana*, *Solen marginatus* and *Ruditapes philippinarum*) in four transects in Mira channel at Ria de Aveiro (40°35′58.30″N, 8°44′47.80″W); *Cerastoderme edule* and *Ruditapes philippinarum* in the Tagus estuary (38°39′27.44″N, 9°6′35.95″W).

2.3. Data and statistical analysis

2.3.1. Data pre-treatment

The relative abundance of each FA was obtained, being calculated as the average and standard deviation for each species and transects. For a better characterization of the FA profiles of the AM of bivalves from different species and transects, FA were grouped according to the number of double bonds they exhibited, SFA, monounsaturated FA (MUFA), PUFA and highly unsaturated FA (HUFA). To reduce the influence of the most abundant FA in the analysis being performed, the relative abundance of each FA was pre-transformed (log(x + 1)).

2.3.2. Comparison of fatty acid signatures of the adductor muscle of different bivalve species from the same ecosystem

A resemblance matrix was calculated based on Bray-Curtis similarity between samples of the six bivalve species sampled from RAv. A permutational analysis of variance (PERMANOVA) was performed to evaluate the existence of significant differences ($p < 0.05$) between factors. The FA profiles of the AM were compared in a two-way crossed model using species as fixed factor with six levels (*C. edule*, *C. gigas*, *M. galloprovincialis*, *R. philippinarum*, *S. plana* and *S. marginatus*) and sampling transects as random factor with four levels (Transects 1–4).

The hierarchical cluster analysis between the FA profiles of the AM of the bivalve species from RAv was performed using agglomerative hierarchical clustering, with the unweighted pair group mean average algorithm (UPGMA). A principal coordinate analysis (PCO) was performed, overlapping vectors of FA that presented Pearson correlation coefficients with the PCO axes higher than 0.85. One-way analysis of variance (ANOVA) was performed to assess significant differences among bivalve species for each individual FA present in their AM, with Tukey's pairwise comparisons being used whenever ANOVA results revealed the existence of significant differences ($p < 0.05$).

All multivariate analyses (PERMANOVA and PCO) were performed using PRIMER v7 with the add-on PERMANOVA+ (Anderson et al., 2008; Clarke and Gorley, 2015), while ANOVAs and Tukey's pairwise comparisons were performed in R environment (R Core Team, 2016).

2.3.3. Comparison of fatty acid signatures of the adductor muscle of the same bivalve species originating from different ecosystems

A resemblance matrix was calculated based on Bray-Curtis similarity between samples of *C. edule* and *R. philippinarum* from RAv and TE. A PERMANOVA was performed to evaluate the existence of significant differences ($p < 0.05$) between the factors. The FA profiles were compared in a two-way crossed model using species (*C. edule* and *R. philippinarum*) and ecosystem (RAv and TE) as fixed factors. Furthermore, samples ordination was evaluated using a PCO. The PERMANOVA and PCO were performed using PRIMER v7 with the add-on PERMANOVA+ (Anderson et al., 2008; Clarke and Gorley, 2015).

3. Results

3.1. Comparison of fatty acid signatures of the adductor muscle of different bivalve species

The average (±SD) relative abundance of each FA recorded for the AM of the six different bivalve species from RAv and two bivalve species from the TE is summarized in Table S2. Thirty-six FA, ranging from 14:0 (myristic acid) to 22:6*n*-3 (docosahexaenoic acid, DHA), were identified among the different species (Table S2). From all FA identified, SFA represented 22–31%, MUFA represented 12–23% and PUFA represented 9–16%. HUFA were the most abundant class of FA recorded in the AM of all bivalve species surveyed, with their levels representing more than 40% of the total pool of FA. The dominant SFA were palmitic (PA, 16:0) and stearic (18:0) acids for all species. The major MUFA was 20:1*n*-9/11 (9,11-eicosenoic acid), which always represented at least 24% of all MUFA recorded in the AM of all bivalve species (Table S2). The second most abundant MUFA was however more species-specific being 18:1*n*-9 (oleic acid) for *S. marginatus*, *R. philippinarum* and *S. plana*, 18:1*n*-7 (vaccenic acid) for *M. galloprovincialis* and *C. edule* from TE and 20:1*n*-7 (paullinic acid) for *C. gigas* and *C. edule* from RAv) (Table S2). The most

dominant PUFA were 22:2*n*-6 (docosadienoic acid) and 22:2*n*-9 for *C. edule*, 22:2*n*-6 (docosadienoic acid) and 18:3*n*-3 (linolenic acid, ALA) for *C. gigas*, 22:2*n*-6 (docosadienoic acid) and 20:2*n*-7 for *M. galloprovincialis*, 22:3*n*-6 and 18:2*n*-6 (linoleic acid, LA) for *S. plana*; 22:2*n*-9 and 22:3*n*-6 for *S. marginatus* and 22:2*n*-6 (docosadienoic acid) and 22:3*n*-6 for *R. philippinarum*. The most abundant HUFA recorded in the AM of all bivalve species surveyed in the present work were 22:6*n*-3 (docosahexaenoic acid, DHA) and 20:5*n*-3 (eicosapentaenoic acid, EPA).

3.2. Comparison of fatty acid signatures of the adductor muscle of bivalve species from the same ecosystem

No significant differences were detected for the interaction transects × species, neither for factor transect. However, significant differences in the FA profiles of the AM were recorded for factor species (PERMANOVA: Pseudo-$F = 99.989$; $p < 0.001$) (Table 1). The pair-wise comparisons revealed significant differences among all bivalve species surveyed (Table 1). The pair-wise comparison that presented the lower *t*-statistics ($t = 5.18$) was that of *C. gigas* with *M. galloprovincialis*.

The hierarchical cluster analysis (Fig. S1) showed two distinct clusters (with 75% similarity), separating the FA profiles of *C. gigas* and *M. galloprovincialis* from the other four bivalve species (*C. edule*, *R. philippinarum*, *S. marginatus* and *S. plana*). Within the latter group, *S. plana* was the first species to be separated, followed by *C. edule* (Fig. S1). The first two axes of the PCO diagram of the bivalve species sampled at RAv explained 65.8% of the FA variation in the data set (PCO axis 1: 45.6%; PCO axis 2: 20.2%; Fig. 2). Along PCO axis 1 the two main cluster groups were separated, while PCO axis 2 separated *S. plana* from other species. Considering each FA individually, *C. gigas* was the single species presenting 18:2*n*-3 and, along with *M. galloprovinciallis*, showed significantly higher values of 18:2*n*-6 (linoleic acid, LA) and 20:5*n*-3 (eicosapentaenoic acid, EPA) (Table S2, Fig. 2, Fig. S2). These species differed significantly between them for 20:5*n*-3 (eicosapentaenoic acid, EPA), but not for 18:2*n*-6 (linoleic acid, LA; Table S2, Fig. 2, Fig. S2). Within the main group of the other four species, *S. plana* presented significantly higher values of 18:0 (stearic acid) and 18:1*n*-9 (oleic acid; Table S1, Fig. 2, Fig. S2). Concerning *C. edule*, this was the only bivalve species whose AM presented 20:2*n*-9 (8,11- eicosadienoic acid; Table S2, Fig. 2). On the other side, 19:0 (margerinic acid) was detected solely in the AM of *S. marginatus*. The levels of 22:5*n*-3 (clupanodonic acid) and 22:6*n*-3 (docosahexaenoic acid, DHA) recorded were significantly higher in the AM of *S. marginatus* and *R. philippinarum*, when compared to the other bivalve species, and did not differ significantly between these two bivalve species (Table S2; Fig. 2; Fig. S2).

Table 1
Results of PERMANOVA main test tests and pair-wise comparison of fatty acid profiles of the adductor muscle of six bivalve species from different transects in Ria de Aveiro. *Cerastoderma edule* (Ce), *Crassostrea gigas* (Cg), *Mytilus galloprovincialis* (Mg), *Ruditapes philippinarum* (Rp), *Scrobicularia plana* (Sp) and *Solen marginatus* (Sm). Significant differences $p < 0.05$.

Main test					
Source	**df**	**SS**	**MS**	**Pseudo-*F***	***p*-value**
Species	5	19,614	3922.7	99.989	0.0001
Transect	3	147.94	49.313	1.0989	0.3541
Species × Transect	15	588.04	39.202	0.8736	0.6912
Res	68	3051.6	44.876		
Total	91	23,534			
Pair-wise comparisons					
Source	**t**	***p*-value**	**Source**	**t**	***p*-value**
Ce vs. Cg	7.2731	0.0001	Cg vs. Sm	12.995	0.0002
Ce vs. Mg	9.6152	0.0001	Mg vs. Rp	10.988	0.0001
Ce vs. Rp	8.7178	0.0001	Mg vs. Sp	9.848	0.0001
Ce vs. Sp	11.117	0.0001	Mg vs. Sm	12.613	0.0001
Ce vs. Sm	8.7122	0.0001	Rp vs. Sp	10.249	0.0001
Cg vs. Mg	5.1773	0.0003	Rp vs. Sm	11.038	0.0001
Cg vs. Rp	12.269	0.0001	Sp vs. Sm	11.436	0.0002
Cg vs. Sp	10.988	0.0001			

3.3. Comparison of fatty acid signatures of the adductor muscle of bivalve species from different ecosystems

A total of 25 FA was identified in the AM of *C. edule* and *R. philippinarum*. The FA 20:2*n*-9 (8,11- eicosadienoic acid) was exclusive of the AM of *C. edule*, while the 18:3*n*-3 (linolenic acid, ALA), 18:4*n*-3 (stearidonic acid), 20:2*n*-6 (dihomo-linoleic acid) and 20:4*n*-3 (eicosatetraenoic acid) were recorded solely in the AM of *R. philippinarum* (Table S2). In general, the most abundant FA were the same for both species originating both from RAv and TE (as shown in 3.1 section).

Samples from RAv presented higher levels of PUFA and lower SFA comparatively to the conspecifics collected from TE (Table S2). Furthermore, specimens from RAv presented higher contents of EPA and 22:6*n*-3 (docosahexaenoic acid, DHA), while those from TE presented higher values of 16:0 (palmitic acid PA), 18:2*n*-6 (linoleic acid, LA) and 18:3*n*-3 (linolenic acid, ALA). While the interaction ecosystem × species was non-significant when comparing the FA profiles of the AM of the bivalve species from the two sampling locations (PERMANOVA, Pseudo-$F = 0.159$, $p = 0.171$), significant differences were recorded for factors ecosystem and species. It is important to highlight that the Pseudo-*F* statistic for factor species (pseudo-$F = 64.400$) was higher than that for factor ecosystem (Pseudo-$F = 12.312$) (Table 2).

The PCO analysis explained 78.4% of the FA variation in the data set (PCO axis 1: 60.9%; PCO axis 2: 17.5%) (Fig. 3). PCO axis 1 showed a clear separation of samples by species (Fig. 3), while PCO axis 2 (Fig. 3) revealed a separation between bivalves originating from RAv and TE. The PCO confirmed that separation by species was clearer than that observed for ecosystems (Fig. 3), in line with the Pseudo-*F* values recorded for PERMANOVA (see above).

4. Discussion

The FA profiles of the AM of bivalves have been successfully used as natural barcodes to trace their geographic origin from locations hundreds of km apart (Grahl-Nielsen et al., 2010; Zhang et al., 2019; Mamede et al., 2020; Ricardo et al., 2017a), as well as from adjacent areas in the same estuarine ecosystem (separated by <10 km) (Ricardo et al., 2015). However, it remained to be investigated whether phylogenetic programming or environmental drivers prevailed on the shaping of FA profiles of the AM of bivalves and how can these drivers be an advantage or constraint for origin certification. The present study suggests, for the first time, that phylogenetic drivers are more powerful than ecosystem-specific environmental drivers in the shaping of the FA profile of the AM of bivalves.

The dominance of 16:0 (palmitic acid PA), 18:0 (stearic acid), 20:5*n*-3 (eicosapentaenoic acid, EPA) and 22:6*n*-3 (docosahexaenoic acid, DHA), as well as PUFA in the FA profiles of the AM recorded for *C. edule*, *C. gigas, Glycymeris nummaria, M. galloprovinciallis* and *R. philippinarum* in the present study, are in accordance with available literature (*C. edule* Ricardo et al., 2017a; Ricardo et al., 2015; *C. gigas* Dridi et al., 2017; *G. nummaria* Najdek et al., 2016; *M. galloprovincialis* Ezgeta-Balić et al., 2012; *R. philippinarum* along the Atlantic western Iberian coast Mamede et al., 2020). Concerning the FA profiles of the AM of *S. plana* and *S. marginatus*, described for the first time in the present study, these species showed similar features with those previously described for other bivalves (*Pecten maximus*, Grahl-Nielsen et al., 2010; *Placopecten magellanicus*, Napolitano et al., 1992, *Astarte sulcata*; Olsen et al., 2009). These results, which refer to bivalve species from five different orders, suggest that the FA profiles of the AM of bivalves do share some general features. Nonetheless, despite these similarities, the FA profiles of the AM of the six species studied in the present work differed significantly, even when collected within the same location. These differences are

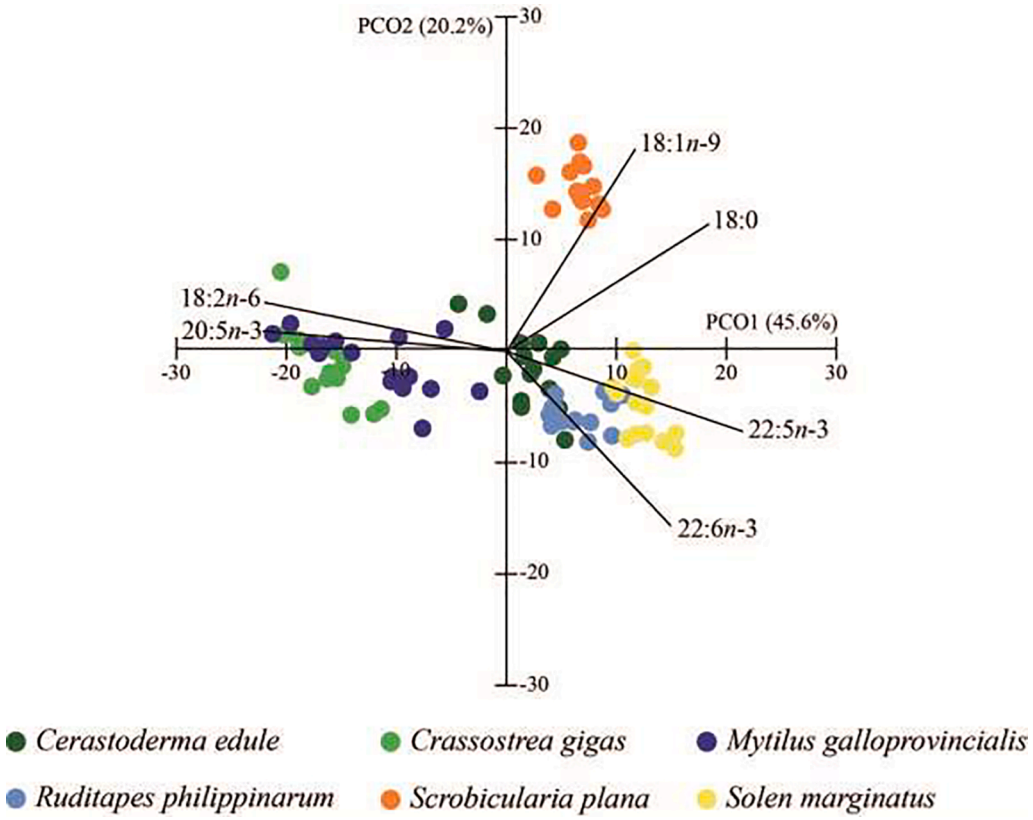

Fig. 2. Principal coordinates analysis (PCO) of fatty acid profiles of the adductor muscle of six bivalve species (*Cerastoderme edule*, *Crassostrea gigas*, *Mytilus galloprovincialis*, *Scrobicularia plana*, *Solen marginatus* and *Ruditapes philippinarum*) from Ria de Aveiro.

Table 2
Results of PERMANOVA main test for fatty acid profiles differences between species and locations. Significant differences $p < 0.05$; n.s., non-significant.

Main test					
Source	**df**	**SS**	**MS**	**Pseudo-*F***	***p*-value**
Species	1	3414.1	3414.1	64.400	0.0001
Location	1	652.7	652.72	12.312	0.0001
Species × Location	1	84.2	1.5878	0.159	0.1713 (n.s.)
Res	36	1908.5	53.013		
Total	39	6059.4			

likely due to intrinsic species-specific and/or phylogenetic features (e.g. type of gills Fillibranch vs. Eulamellibranch; (Gosling, 2008)). Indeed, diet quality/biochemical profile affects the FA pool displayed by bivalves, as different species exhibit different trophic spectra (in terms of particles size being ingested and their biochemical composition) that promote contrasting FA profiles (Ezgeta-Balić et al., 2012). As FA *de novo* synthesis and metabolism may vary among taxa, a direct inference between the FA profile of a given organism and that of its diet may be unclear (Happel et al., 2016). Kraffe et al. (2008) investigating the FA profiles of mitochondrial cardiolipins, and Makhutova et al. (2011) addressing diverse freshwater benthic invertebrates, found evidence that phylogenetic drivers play a major role in the FA profiles displayed by organisms colonizing similar environments. Indeed, species phylogenetically more closely related are expected to display more similar FA profiles than those with greater phylogenetic distances. In the present study, this trend was evidenced with the separation of *C. gigas* and *M. galloprovincialis* (Infraclass Pteriomorphia) from the rest of the bivalve species surveyed (Infraclass Heteroconchia) (WoRMS Editorial Board, 2020). However, these trends are not straight forward, as the two most closely related species studied, from a phylogenetic point of view (both within the same Order, Cardiida), *C. edule* and *S. plana*, are not the ones displaying the most similar FA profiles on their AM. Indeed, one cannot simply rule out the role played by food availability or environment-driven physiological status (e.g. driven by water salinity and temperature) of species within the same habitat (Zhukova, 2019).

The FA profiles of the AM of *C. edule* and *R. philippinarum* from RAv and TE displayed significant differences between species and ecosystems. Differences between FA profiles of the AM of *C. edule* and *R. philippinarum* from RAv and TE were already reported by Ricardo et al. (2017a) for *C. edule*, and by Mamede et al. (2020) for *R. philippinarum*. These two ecosystems display contrasting physical–chemical conditions and productivity, which is known to affect the FA composition of marine organisms (Calado and Leal, 2015; Dalsgaard et al., 2003; Zhukova, 2019). Specimens from RAv presented higher levels of PUFA when compared to conspecifics sampled in TE, which suggest that bivalves in RAv may be more exposed to cooler waters, favoring higher levels of PUFA (Fokina et al., 2015; Nemova et al., 2013). Furthermore, the higher contents of 22:6*n*-3 (docosahexaenoic acid, DHA) in the AM of specimens from RAv suggest a prevalence of a dinoflagellate based diet, while higher contents of 18:2*n*-6 (linoleic acid, LA) and 18:3*n*-3 (linolenic acid, ALA) in the specimens from TE point towards a higher ingestion of green microalgae when compared to conspecifics from RAv (Dalsgaard et al., 2003). Overall, it must be highlighted that the differences recorded between bivalve species are higher than those displayed between ecosystems.

5. Conclusion

The present study represents a step forward towards the use of FA profiles of the AM as natural barcodes to trace the place of origin of commercially important bivalves. Although bivalves from the same location present some similarities in their FA signatures, there is a significant species-specific variability that must be taken into account.

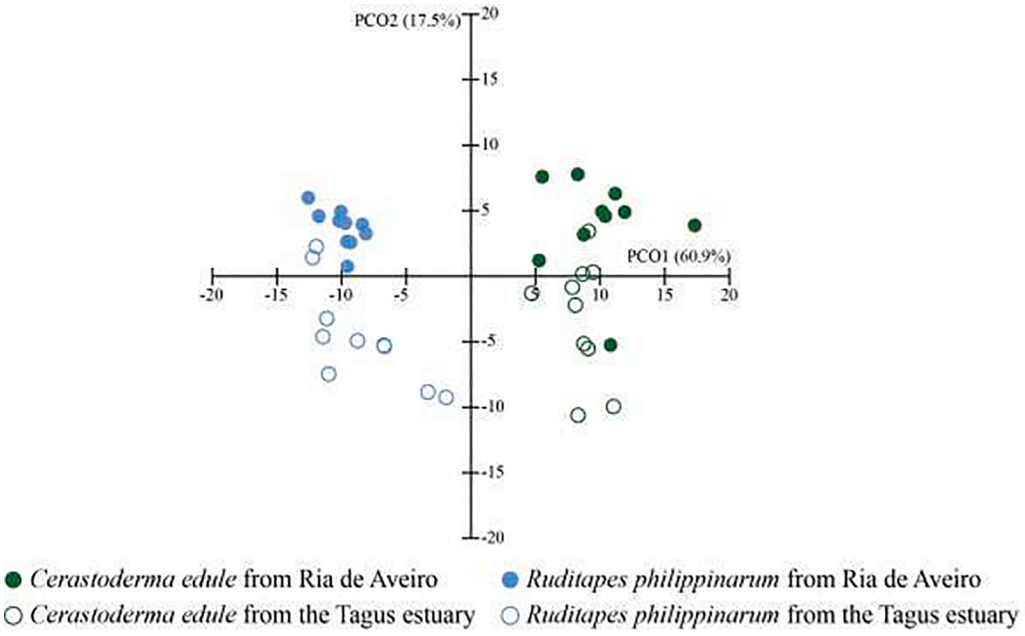

Fig. 3. Principal coordinates analysis (PCO) of fatty acid profiles of the adductor muscle of two bivalve species (*Cerastoderma edule and Ruditapes philippinarum*) from Ria de Aveiro and the Tagus estuary.

Indeed, the present study suggests that phylogenetic drivers prevail over ecosystem-specific environmental drivers in the build-up of FA signatures in the AM of bivalves. At present, this finding discourages the use of a reference model developed for a given bivalve species to be used to determine the geographic origin of another one, even if both are closely related and originate from the same location.

CRediT authorship contribution statement

Fernando Ricardo: Conceptualization, Investigation, Writing – original draft, Writing - review & editing, Visualization. **Diana Gonçalves:** Methodology, Writing - review & editing. **Tânia Pimentel:** Methodology, Writing - review & editing. **Renato Mamede:** Methodology, Writing - review & editing. **M. Rosário M. Domingues:** Conceptualization, Investigation, Writing – original draft, Writing - review & editing, Supervision. **Ana I. Lillebø:** Writing - review & editing, Project administration, Funding acquisition. **Ricardo Calado:** Conceptualization, Investigation, Writing – original draft, Writing - review & editing, Project administration, Funding acquisition, Supervision.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.108017.

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