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Spatio-temporal variability in the fatty acid profile of the adductor muscle of the common cockle *Cerastoderma edule* and its relevance for tracing geographic origin

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ABSTRACT

The present study aimed to identify the potential existence of spatio-temporal variability in the fatty acid (FA) profile of the adductor muscle (AM) of a commercially important bivalve, the common cockle *Cerastoderma edule*, and determine if such variability can be used to trace their geographic origin post-harvesting. Common cockles were sampled in eight ecosystems along the coast of mainland Portugal, as well as in two different channels within one of those ecosystems over two consecutive years. Results showed significant differences in FA profiles among ecosystems, namely due to different levels of eicosapentaenoic acid (EPA; 20:5*n*-3), arachidonic acid (20:4*n*-6), ratio of polyunsaturated FA (PUFA) *n*-3/*n*-6, monounsaturated FA (MUFA; 18:1*n*-9 and 20:1*n*-9/11) and bacterial FA (15:0, 17:0 and 18:1*n*-7). FA profiles also displayed significant differences between two consecutive years in channels from the same ecosystem. Overall, while the FA profile displayed by the AM of *C. edule* can be successfully used to trace their geographic origin, the existence of temporal variability requires a periodical verification of FA signatures to identify potential shifts, namely when comparing specimens from the same ecosystem.

1. Introduction

The spatial distribution of bivalves in marine and estuarine ecosystems is shaped by a number of environmental variables, such as temperature, salinity and sediment type, as well as by food availability (Gosling, 2004). As environmental and trophic history of bivalves significantly condition their fatty acid (FA) profiles (Caers, Coutteau, & Sorgeloos, 2000; Calado & Leal, 2015; Prato, Danieli, Maffia, & Biandolino, 2010), these molecules have received much attention in environmental (Prato et al., 2010) and trophic ecology studies (Dalsgaard et al., 2003) in the marine environment. Indeed, salinity and temperature are known to modulate the structure and fluidity of cell membranes, with higher saline fluctuations and/or lower water temperatures promoting a decrease in the levels of saturated FA (SFA), which are responsible to stabilize the bilayer structure, and an increase in the concentration of polyunsaturated FA (PUFA), which enhance the bilayer fluidity (Nemova, Fokina, Nefedova, Ruokolainen, & Bakhmet, 2013). Moreover, several FA may reveal the dietary role that specific taxonomic groups may play in marine food webs (Bergé & Barnathan, 2005; Dalsgaard et al., 2003; Galap, NetchitaïLo, Leboulenger, & Grillot, 1999). For instance, while high contents of 18:4*n*-3 in marine animal tissues often indicate a diet dominated by dinoflagellates (Dalsgaard et al., 2003; Ezgeta-Balić, Najdek, Peharda, & Blažina, 2012) and an enrichment in 16:1*n*-7 may reveal a diet mainly supported by diatoms (Parrish et al., 2000), significant contents of odd chain fatty acids (e.g. 15:0, 17:0) or 18:1*n*-7 are often related to the consumption by bacteria (Bergé & Barnathan, 2005; Dalsgaard et al., 2003). These inferences are possible by the fact of several marine organisms, such as bivalves, being unable to synthesize these molecules *de novo*, thus having to rely on their dietary items to fulfil their needs and selectively retaining these molecules in their tissues (Calado & Leal, 2015; Dalsgaard et al., 2003.)

In the global seafood market, bivalve, clams, scallops, mussels and cockles play the key-role, as they support commercial fisheries and aquaculture production worldwide (Telahigue, Chetoui, Rabeh, & Romdhane, 2010). The common cockle (*Cerastoderma edule*) is

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one of the most commonly harvested bivalves in Europe, with a production of 26 125 tonnes in 2015 (FAO, 2016). In Portugal, this species tops the rank of commercially harvested molluscs, with its captures having considerably increased in recent years; in 2015 alone, over 5.000 tonnes have been harvested, representing an estimated revenue of 4.5 million € (INE, 2015). With such high commercial relevance, it is of little surprise to verify that fraudulent practices employing the trade of poorer quality specimens have also emerged. The most common form of fraud is the trade of specimens collected in microbiological contaminated/environmentally polluted waters unsuitable for bivalve production, that ultimately represent a threat to public health, as originating from certified production areas (Maalouf, Pommepuy, & Le Guyader, 2010). With the growing complexity of trade-chains and fraudulent schemes, the geographic origin of traded seafood in general, and bivalves in particular, deserves an unprecedented level of attention by consumers (Leal, Pimentel, Ricardo, Rosa, & Calado, 2015; Ricardo et al., 2015, 2017). Hence, reliable tools to trace the geographic origin of bivalves are of paramount importance to ensure their quality and safety, and allow the enforcement of current regulations concerning seafood traceability already in place (e.g. EC, 2002).

The adductor muscle (AM) of bivalves as emerged as a suitable matrix to study FA signatures, as this specific organ displays lower FA turnover rates promoted by short-term trophic and environmental variability (Dalsgaard et al., 2003). The composition of lipids present in the AM of bivalves is dominated by phospholipids and sterols, thus providing a FA signature that is mainly related with environmental conditions, such as water temperature, water depth and salinity (Caers et al., 2000; Prato et al., 2010), than short term shifts in dietary regimes (Dalsgaard et al., 2003; Napolitano, Pollero, Gayoso, Macdonald, & Thompson, 1997). Trophic-driven shifts in the FA profile of the AM only occur after long periods of exposure to a specific dietary item/group, hence reflecting prevalent trophic conditions (Paulet, Lorrain, Richard, & Pouvreau, 2006). The FA profiles displayed by the AM of certain bivalve groups (e.g. scallops and cockles) has already been used to evidence significant differences in specimens originating from areas up to 100s of km apart (Olsen, Grahl-Nielsen, & Schander, 2009), as well as from adjacent areas (<10 km apart) within the same estuary/coastal lagoon (Ricardo et al., 2015). Besides, the latter study also showed that FA profiles of the AM can be used to discriminate, within the same coastal system, bivalves originating from capture/production areas with different microbiological safety levels as detailed in Council Regulation 853/2004 and 854/2004 (EC, 2004a, 2004b).

In order to successfully use the FA profile of the AM of bivalves to discriminate their geographic origin it is important to investigate the existence of any potential temporal variability and if it may bias the discrimination power of this approach. The present study aimed to verify if the FA profile of the AM of *C. edule* could be successfully employed to discriminate the geographic origin of fresh specimens harvested in eight different ecosystems along the Atlantic coast of mainland Portugal and evaluate if temporal variability affected the FA profile of the AM over two consecutive years in two areas of one of the study locations.

2. Material and methods

2.1. Study areas and cockle collection

A total of 100 samples of *C. edule* with a shell length >25 mm (commercial size, approximately 3 years old) (Seed & Brown, 1978) were collected during June–July of 2014 from eight different Portuguese ecosystems where this species is commercially explored: Ria de Aveiro (RAv), Óbidos lagoon (OL), Tagus estuary (TE), Albufeira lagoon (AL), Sado estuary (SE), Mira estuary (ME), Ria do Alvor (RAl) e Ria Formosa (RF) (Fig. 1). In RAv three of the main channels (Mira, Ílhavo and Espinheiro) of this coastal lagoon were sampled. Two areas were surveyed in each channel (Fig. 1a) and five replicates were collected per area (3 channels X 2 areas X 5 replicates = 30 samples). For OL, TE, AL, SE and RF two areas were surveyed with five cockles being collected per area (5 ecosystems X 2 areas X 5 replicates = 50 samples). In RAl and ME due to few abundance of cockles, were collected ten specimens in just one area (2 ecosystems X 1 areas X 10 replicates = 20 samples).

In order to evaluate the potential existence of temporal variability in the FA profile of the AM, specimens of *C. edule* from Ílhavo (I) and Espinheiro (E) channels at RAv were collected exactly in the same locations in June 2013 and June–July 2014 for comparison. Five specimens were collected per area in these two consecutive years (1 ecosystem X 2 channels X 2 areas X 2 years X 5 replicates = 40 samples). Bivalves were surveyed during the summer time, as the consumption of this highly priced seafood significantly increases during this period and thus with increasing demand and higher market values fraudulent practices are more prone to occur.

All samples were collected manually with the help of a hand-rake and stored in aseptic plastic bags. After collection, cockles were kept refrigerated and transported to the laboratory and stored at −20 °C for further processing. All collected specimens were dissected to extract the AM, which were freeze-dried then stored at − 80 °C for subsequent FA analysis.

2.2. Fatty acids analysis

Total lipids from the AM of each individual cockle were quantified through gravimetric method after extraction following the procedure described by Bligh and Dyer (1959). Methyl esters of FAs (FAME) were prepared according to Aued-Pimentel, Lago, Chaves, and Kumagai (2004) method (by transmethylation of FA using a mixture of methanolic solution KOH (2 M) and satured NaCl). Gas chromatography–mass spectrometry (GC–MS) analysis were performed using an Agilent Technologies 6890 N Network (Santa Clara, CA) equipped with a DB-FFAP column with 30 m of length, 0.32 mm of internal diameter, and 0.25 μm of film thickness (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 in a 1 s cycle in a full scan mode acquisition. The oven temperature was programmed from an initial temperature of 80 °C, with a linear increase to 220 °C being performed at 14.4 °C min^{-1}, followed by a linear increase at 10 °C min^{-1} to 240 °C and 5 °C min^{-1} to 250 °C. The injector and detector temperatures were 220 and 280 °C, respectively. Helium was used as the carrier gas at a flow rate of 0.5 mL min^{-1}. Individual FAs peaks were identified by comparing the retention time and mass spectra of each FA relative to 34 mixed FA standards (C4–C24, Supelco 37 Component Fame Mix), and confirmed by comparison with the spectral library "The AOCS Lipid Library" (AOCS, 2012).

2.3. Statistical analysis

In order to select the best subset of variables that may explain potential differences between the different areas sampled within each ecosystem, FAs were grouped by classes as saturated FA (SFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA). Within each class, FAs displaying the same pattern were pooled to simplify further statistical analysis. A preliminary multivariate analysis of variance (MANOVA; Table S1 on supplementary data) was performed to detect significant differences in the FA profile displayed by the AM of *C. edule* sampled in different areas of the