Ecological Indicators 124 (2021) 107378

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

# Assessment of seasonal and spatial variations in the nutritional content of six edible marine bivalve species by the response of a set of integrated biomarkers

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ARTICLE INFO

*Keywords:*
Bivalves
Economic value
Biochemical biomarkers
Nutritional value
Mondego estuary
Ria Formosa lagoon

ABSTRACT

Bivalves are widely distributed through diverse habitats, including estuaries and coastal lagoons which are extremely productive ecosystems, and play important roles in trophic webs and in ecosystems' biological processes. Bivalves, as well as other marine resources, have been a part of the humans' diet since mankind started fishing. These resources have high nutritional values, being constituted by high protein and low fat contents, and its consumption is associated with several health benefits. Marine resources, like bivalves, that are highly appreciated by humans, represent an important economic value, being under pressure due to an increasing demand. Thus, it is important a sustainable and balanced exploitation of these resources, based on the knowledge of the biochemical composition of the aquatic species to comprehend its' potential and nutritional value.

The present study was conducted in Portugal, a country that has one of the highest consumptions of seafood in the world. Six commercially valuable species of marine bivalves were harvested in two distinct areas, Mondego estuary and Ria Formosa lagoon, and in two seasons, winter 2016 and summer 2017. The aims of the study were to: 1) determine the biochemical composition of each species in terms of total protein content, fatty acid and carbohydrate profiles; 2) identify potential spatial and seasonal variations between bivalve species sampled in each study area and season; 3) assess feeding behaviour of the bivalve species in both seasons and study areas.

The results indicated diverse biochemical composition among bivalve species, with total protein as the major component, followed by fatty acid content, particularly by the essential fatty acids DHA and EPA, and glycogen and glucose as the main polysaccharide and monosaccharide, respectively, found in all specimens. In general, all species demonstrated a tendency for omnivory, with only *S. marginatus* presenting a clear herbivorous behaviour in summer. Despite *M. galloprovincialis* and *R. decussatus* showed the highest nutritional value in the Mondego estuary, in both seasons, it was more noticeable in winter. In Ria Formosa, *C. edule* and *R. decussatus* showed the highest nutritious value in both seasons, while *C. gigas* showed higher nutritive value in summer.

## 1. Introduction

Marine ecosystems, in particular, estuaries and coastal lagoons, are among the most important environments in the world, revealing unique features such as high productivity and biodiversity of species, as well as the existence of key-species, ecosystem services and, consequently, the existence of valuable marine resources to human-beings (McLusky and Elliott, 2004; Barbier et al., 2011; Ahmed et al., 2014). Bivalve species (phylum Mollusca) play important roles in the trophic web and in the ecosystem's structure and processes, being related to several ecosystem services. Bivalves are among the major preys of gastropods, starfish, crabs, fish, birds, and mammals, and obtain, through filter-feeding or suspension-feeding, substantial amounts of suspended material from the water (Dame, 2012; Gonçalves et al., 2016). Phytoplankton (diatoms and dinoflagellates) is their primary food source, but traces of zooplankton, bacteria and detritus can be found in their tissues (Prato

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https://doi.org/10.1016/j.ecolind.2021.107378
Received 7 October 2020; Received in revised form 6 January 2021; Accepted 8 January 2021
Available online 30 January 2021

et al., 2010; Ezgeta-Balić et al., 2012). These organisms have a wide geographical distribution, including transitional waters systems (e.g. estuaries) and coastal waters systems (e.g. coastal lagoons) (Gosling, 2003). Due to their importance in the ecosystem, any anthropogenic or natural perturbation may affect the physiological processes, behaviour and mortality of bivalves, which have a direct impact on the trophic food web and in the ecosystem functioning (Fuji, 2012; Verdelhos et al., 2015).

Bivalves have been included in our dietary patterns since our ancestors, who lived nearby coastal regions and other water bodies, started to fish and gather these resources (Colonese et al., 2011). Bivalves and other seafood are associated with high nutritional value, being constituted by high protein and low fat contents, carbohydrate contents, essential amino acids and fatty acids, vitamins, minerals, and trace elements (Larsen et al., 2011; Tacon and Metian, 2013). Glucose is stored in the bivalves' tissues in the form of glycogen, the main polysaccharide with an important storage role (Matias et al., 2013). Proteins are the major constituents of bivalves, assuming structural and energy storage functions when glycogen levels are low (de Zwaan and Zandee, 1972; Pérez Camacho et al., 2003). In terms of fatty acid (FA) profile, bivalves have saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs), which include monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Highly unsaturated fatty acids (HUFAs), an important subset of the last group that comprise arachidonic acid (ARA or C20:4n-6), eicosapentanoic acid (EPA or C20:5n-3), and docosahexanoic acid (DHA or C22:6n-3), are fatty acids that cannot be synthesised *de novo* by bivalves. Therefore, these fatty acids are acquired through dietary input and are considered essential fatty acids. Other essential fatty acids that can be found in bivalves' biochemical composition are α-linolenic acid (ALA or C18:3n-3) and linoleic acid (LA or C18:2n-6) (Ezgeta-Balić et al., 2012; Gonçalves et al., 2016, 2017a, 2017b). The consumption of essential fatty acids from seafood have several beneficial properties to human health, including good development of the nervous and immune systems in infants and reduction of the incidence of cardiovascular diseases (Riediger et al., 2009; Larsen et al., 2011). Furthermore, fatty acids are considered good bioindicators of environmental and chemical stress (Gonçalves et al., 2012). In previous studies, changes in the bivalves' biochemical composition were associated with their state of sexual maturity, the energy supply provided either by food ingestion or by previously stored reserves, and the environmental surroundings (Pérez Camacho et al., 2003; Aru et al., 2017).

Bivalves are one of the main seafood products consumed and traded around the world (Tacon and Metian, 2013). Oysters, mussels, scallops and clams are the most traded bivalves worldwide and represent a high commercial value (FAO, 2018b). The increasing consumption of bivalves by humans and the consequent intensification of overexploitation, allied with climate change, invasive species and coastal development represent major threats to the conservation of highly valuable bivalve species. Thus, to prevent the collapse of these species, the consumption needs to be balanced with the sustainability and good management of the species stocks (Kearney, 2010; Almeida et al., 2015).

The major seafood markets in the world are in Asia, America and Europe (Swartz et al., 2010). For a while, China has been the main producer of bivalves (75.32% of the global production of bivalves in 2013), followed by Japan (4.84% in 2013) and the United States of America (4.29% in 2013) (FAO, 2016, 2017, 2018a). Regarding European countries, France and Spain are the top producers of bivalves. Nevertheless, Portugal is an important contributor to the total European bivalve production and the 3rd country with the highest seafood consumption *per capita* (53.76 kg in 2013) (FAO, 2016, 2017). Among the most valuable and traded bivalve species captured and harvested in Portugal are the Grooved carpet shell *Ruditapes decussatus* (2344 t in 2016), the Common cockle *Cerastoderma edule* (1958 t in 2016), the Pacific cupped oyster *Crassostrea gigas* (634 t in 2016), the Mediterranean mussel *Mytilus galloprovincialis* (373 t in 2016), the Grooved razor shell *Solen marginatus* (171 t in 2016) and the Peppery furrow shell *Scrobicularia plana* (<1t in 2016) (FAO, 2018a). These species are produced in several regional coastal areas of Portugal, including the Mondego estuary, a transitional water system located in the northwest coast near Figueira da Foz city, and the Ria Formosa lagoon, a coastal system situated in the south coast, and these were the selected species for the present study.

Considering the central role of bivalve molluscs in the transitional waters and coastal ecosystems and in the marine food webs, as well as their economic value for humans and the environmental conditions during seasonal changes at the Portuguese coast, it is crucial to determine and assess the biochemical composition of most consumed species from different coastal areas and different seasons, in order to evaluate the impact of spatial and seasonal changes in this composition. Therefore, this study aimed to 1) determine the biochemical profiles of six valuable bivalve species sampled in two distinct geographic areas from Portugal (Mondego estuary and Ria Formosa lagoon) and in two seasons (winter and summer), 2) identify seasonal and spatial variations of the biochemical profiles and 3) assess feeding behaviour of the studied bivalve species by determining fatty acid trophic markers.

## 2. Materials and methods

### 2.1. Studied areas

The Mondego estuary is located near Figueira da Foz city (40°08′ N, 8°50′ W) and is a mesotidal system covering an area of 8.6 $km^2$ along the West Atlantic coast (Fig. 1A). It comprises two channels, north and south, separated by the Murraceira island, that join again near the mouth. The north channel is deeper (4–8 m in high tides; tidal range 1–3 m), being mainly used as a navigation channel, and more hydrodynamic than the south channel. The south channel is shallower (2–4 m in high tides; tidal range 1–3 m) and, therefore, the water flow depends on the tides and freshwater input from the Mondego river and its main tributary, Pranto river. The discharge from this tributary is influenced by a sluice that is regulated by the rice field farmers of the Lower Mondego Valley (Martins et al., 2001; Marques et al., 2003; Lillebø et al., 2005; Teixeira et al., 2008; Gonçalves et al., 2016). Recent data demonstrate a salinity variation between 22.1 and 39. The water temperature rages between 11.4 °C in winter and 21 °C in summer (D'Ambrosio et al., 2019; Vieira et al., 2018).

The Ria Formosa lagoon is located in the south coast of Portugal (36°58′ N, 8°02′ W to 37°03′ N, 7°32′ W) and is a shallow mesotidal system composed by multiple channels, salt marshes and tidal flats, covering an area of approximately 84 $km^2$ (Fig. 1B). Sandy barrier-islands protect this system from the Atlantic Ocean. The mean depth does not usually surpass 3 m. The tide amplitude fluctuates between 1.3 and 3.5 m in neap and spring tides, respectively. The tides have a stronger impact in this system than the input of freshwater that comes from several intermittent rivers and streams and, as a result, the salinity values demonstrate an oscillation between 35.5 and 36.9. This range suffers a brief oscillation in surface waters when there are heavy winter rainfall periods. The water temperature rages between 12 °C and 27 °C in winter and summer seasons, correspondingly (Ribeiro et al., 2008; Cravo et al., 2012; Guimarães et al., 2012).

The Mondego estuary and the Ria Formosa lagoon are ecosystems that have high productivity and high biodiversity, including flora and fauna that are found specifically in these ecosystems. Both areas provide important resources to the human populations, including fisheries, industries, agriculture, salt production and tourism (Marques et al., 2003; Almeida and Soares, 2012).

### 2.2. Environmental characterization

2016 was a warm year, with a mean annual air temperature (15.91 °C) higher than the normal climatic value of 1971–2000 (+0.65 °C). 5 heat waves occurred in this year (3 in summer and 2 in

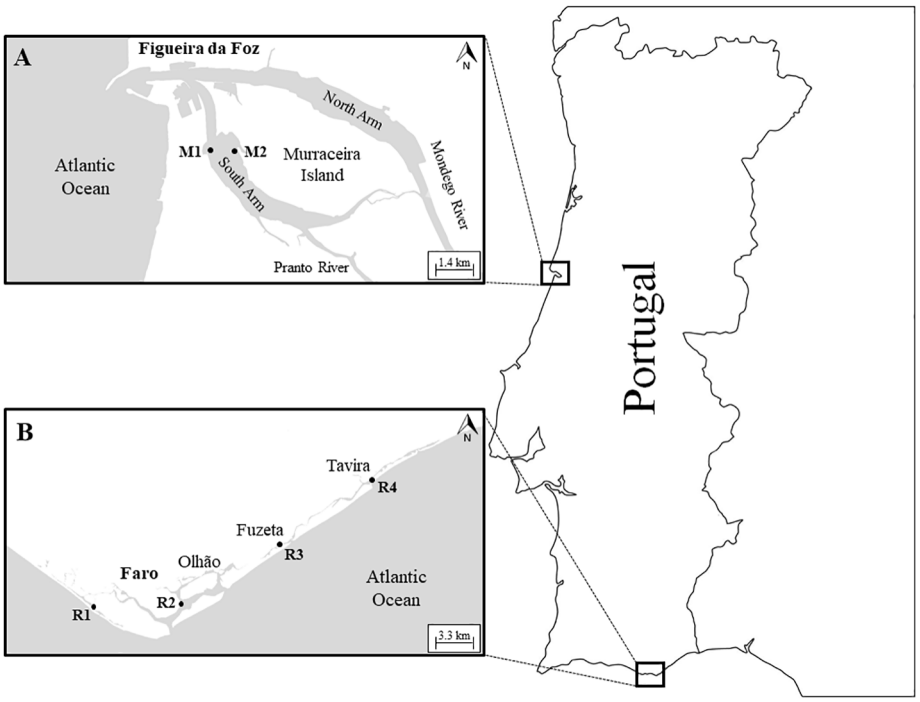


**Fig. 1.** Bivalve species sampled in two distinct studied areas from the Portuguese coast: the Mondego estuary (A) and the Ria Formosa lagoon (B). Black dots represent the sampling stations: M1 (*M. galloprovincialis*) and M2 (*C. edule*, *R. decussatus, S. plana* and *S. marginatus*) in the Mondego estuary, R1 (*S. marginatus*), R2 (*M. galloprovincialis*), R3 (*C. edule* and *R. decussatus*) and R4 (*C. gigas*) in the Ria Formosa lagoon.

autumn). The annual total precipitation of 991.6 mm was above the normal climatic value of 1971–2000 (+109.5 mm). According to Instituto Português do Mar e da Atmosfera (2016), 2016 was the 11th warmer year since 1931.

2017 was considered an extremely warm and driest year. In fact, it was the 2nd warmer year since 1931, with a mean annual air temperature (16.33 °C) above the normal climatic value of 1971–2000 (+1.07 °C). 1 cold wave happened in January and 7 total heat waves occurred in this year (2 in spring, 2 in summer and 3 in autumn). The annual total precipitation of 541.3 mm was the 3rd lowest year since 1931, according to Instituto Português do Mar e da Atmosfera (2017).

### 2.3. Sampling collection

Sampling campaigns occurred in winter (December of 2016) and in summer (June of 2017). Three replicates per species were randomly harvested to be used in each one of the biochemical analysis, except for total protein content analysis where six replicates were used. In the Mondego estuary, the organisms were harvested in the south channel during low tide. *Mytilus galloprovincialis* was sampled in the sampling station M1, while *C. edule*, *R. decussatus*, *S. plana* and *S. marginatus* were collected in the sampling station M2 on the opposite margin (Fig. 1A). Additionally, in the case of *M. galloprovincialis,* adults with different sizes (S: small size; B: big size) were sampled in winter and summer seasons, to evaluate any biochemical change related to the size variation of the species. Due to low abundance in the estuary, the replicates of *R. decussatus* and *S. marginatus* were divided equally into sub-replicates to have enough samples to proceed to biochemical analysis. In the Ria Formosa lagoon, *C. edule* and *R. decussatus* (sampling station R3 in Fig. 1B), *C. gigas* (sampling station R4), *M. galloprovincialis* (sampling station R2) and *S. marginatus* (sampling station R1) were harvested by artisanal fisherman's and brought to the lab in the University of Algarve, where the samples were processed. Since *C. gigas* is a species with higher economic value in the south of Portugal, it was sampled instead of *S. plana*. After harvest, samples were divided by species, immediately reserved inside cold boxes (4 °C) and transported to the lab. During the sample processing biometric parameters were registered, including soft tissue weight, total weight (with valves), length and width of the valves (Table 1). Condition index (CI) was accessed as the ratio of wet soft tissue weight/total wet weight (Lemaire et al., 2006). The edible portion of each bivalve sample was stored and preserved at −80 °C until the biochemical analysis.

### 2.4. Biochemical analysis

#### 2.4.1. Fatty acid analysis

The edible portion of the bivalve (soft tissue) was entirely used in the fatty acid extraction analysis. The total lipid extraction and methylation to fatty acid methyl esters (FAMEs) was achieved by a modified one-step derivatisation method as described by Gonçalves et al. (2012). The boron trifluoride-methanol reagent was replaced by a 2.5% $H_2SO_4$-methanol solution since $BF_3$-methanol can cause artefacts or loss of polyunsaturated fatty acids (PUFAs) (Eder, 1995). FAMEs present in the samples were separated and quantified using a Agilent 6890 N Network Gas Chromatograph (Agilent Technologies, Santa Clara, CA, USA), equipped with a DB-FFAP capillary column (30 m long × 0.32 mm i.d. × 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA), associated to a 5973 N Mass Selective Detector (Agilent Technologies, Santa Clara, CA, USA) at 70 eV electron impact mode, scanning the range *m/z* 40–500 in 1 s cycle in full scan mode acquisition. The carrier gas He had a 4.4 mL $min^{-1}$ flow rate and 2.66 psi of column head pressure. 1 μL of sample was injected per run at the injector port, at a temperature of 250 °C, lined with a splitless glass liner of 4.0 mm i.d. Each run had a 42.53 min duration. The injection temperature was 220 °C and the oven temperature was programmed to start at 80 °C, increase to 160 °C at a 25 °C $min^{-1}$ rate, increase to 210 °C at a 2 °C $min^{-1}$ rate, increase to 250 °C $min^{-1}$ at a 30 °C $min^{-1}$ rate and finally maintaining this temperature for 10 min. The detector starts operating 4 min after injection, corresponding to solvent delay. The injector ion source and transfer line were maintained at 220 °C and 250 °C, respectively. FAMEs were identified by comparison with the retention times and mass spectra of authentic standards and database available

**Table 1**
Biometric parameters measured (mean ± standard error) during the sample processing and respective sample size (n = number of organisms) of the bivalve species from the Mondego estuary and the Ria Formosa lagoon. *S means small organisms; **B means big organisms.

| | | Species | Sample size (n) | Total weight (g) | Soft tissue weight (g) | Condition Index (CI) | Height (mm) | Length (mm) |
|---|---|---|---|---|---|---|---|---|
| Mondego estuary | Winter | *C. edule* | 15 | 12.52 ± 0.69 | 2.71 ± 0.14 | 0.22 ± 0.00 | 36.69 ± 0.34 | 41.57 ± 0.32 |
| | | *M. galloprovincialis* (S*) | 15 | 0.80 ± 0.05 | 0.23 ± 0.02 | 0.28 ± 0.01 | 16.29 ± 0.26 | 31.57 ± 0.33 |
| | | *M. galloprovincialis* (B**) | 15 | 3.43 ± 0.12 | 1.21 ± 0.05 | 0.35 ± 0.00 | 26.51 ± 0.42 | 52.67 ± 0.80 |
| | | *R. decussatus* | 3 | 28.18 ± 7.53 | 6.60 ± 2.00 | 0.23 ± 0.01 | 52.07 ± 3.07 | 36.80 ± 1.85 |
| | | *S. plana* | 15 | 3.34 ± 0.17 | 1.14 ± 0.06 | 0.34 ± 0.01 | 25.93 ± 0.42 | 42.93 ± 0.31 |
| | | *S. marginatus* | 5 | 15.95 ± 2.19 | 10.64 ± 1.36 | 0.67 ± 0.01 | 90.16 ± 2.17 | 18.16 ± 1.07 |
| | Summer | *C. edule* | 15 | 12.83 ± 0.86 | 2.83 ± 0.26 | 0.22 ± 0.01 | 32.99 ± 0.69 | 36.74 ± 0.81 |
| | | *M. galloprovincialis* (S*) | 15 | 2.14 ± 0.14 | 0.69 ± 0.06 | 0.32 ± 0.01 | 20.50 ± 0.46 | 37.37 ± 0.82 |
| | | *M. galloprovincialis* (B**) | 15 | 8.60 ± 0.24 | 3.50 ± 0.10 | 0.41 ± 0.01 | 32.99 ± 0.45 | 62.41 ± 0.74 |
| | | *R. decussatus* | 3 | 21.67 ± 2.42 | 5.50 ± 0.29 | 0.26 ± 0.02 | 35.93 ± 1.37 | 47.43 ± 4.10 |
| | | *S. plana* | 15 | 5.40 ± 0.35 | 1.70 ± 0.14 | 0.32 ± 0.01 | 33.27 ± 0.65 | 42.56 ± 0.90 |
| | | *S. marginatus* | 15 | 12.37 ± 0.33 | 8.03 ± 0.25 | 0.65 ± 0.01 | 15.35 ± 0.17 | 96.84 ± 1.04 |
| Ria Formosa lagoon | Winter | *C. edule* | 15 | 4.43 ± 0.19 | 0.95 ± 0.04 | 0.22 ± 0.01 | 23.86 ± 0.31 | 28.06 ± 0.37 |
| | | *C. gigas* | 15 | 58.58 ± 2.93 | 6.75 ± 0.54 | 0.12 ± 0.01 | 46.90 ± 1.72 | 88.82 ± 2.28 |
| | | *M. galloprovincialis* (B**) | 15 | 10.83 ± 0.64 | 2.35 ± 0.16 | 0.22 ± 0.01 | 28.39 ± 1.87 | 59.27 ± 1.06 |
| | | *R. decussatus* | 15 | 8.40 ± 0.45 | 2.14 ± 0.15 | 0.25 ± 0.01 | 27.69 ± 0.48 | 39.44 ± 0.65 |
| | | *S. marginatus* | 15 | 8.65 ± 0.54 | 5.25 ± 0.32 | 0.61 ± 0.01 | 14.57 ± 0.28 | 86.66 ± 2.12 |
| | Summer | *C. edule* | 15 | 6.27 ± 0.27 | 0.95 ± 0.06 | 0.15 ± 0.00 | 27.42 ± 0.30 | 31.83 ± 0.43 |
| | | *C. gigas* | 15 | 62.60 ± 2.86 | 10.55 ± 0.79 | 0.17 ± 0.01 | 51.27 ± 1.09 | 88.96 ± 1.54 |
| | | *M. galloprovincialis* (B**) | 15 | 15.37 ± 0.94 | 3.17 ± 0.24 | 0.21 ± 0.01 | 33.65 ± 0.75 | 66.18 ± 1.31 |
| | | *R. decussatus* | 15 | 13.34 ± 0.80 | 3.22 ± 0.20 | 0.24 ± 0.01 | 32.75 ± 0.63 | 43.72 ± 0.83 |
| | | *S. marginatus* | 15 | 13.02 ± 0.61 | 7.36 ± 0.35 | 0.57 ± 0.01 | 16.50 ± 0.19 | 97.66 ± 1.24 |

(WILEY Mass Spectral Libraries). Quantification of individual FAMEs was accomplished using an external standard (Supelco™ 37 Component FAME Mix, Supelco#47885, Sigma-Aldrich Inc., USA).

#### 2.4.2. Total protein content

Bivalves' body tissue from each sample was weighted (~60 mg), thawed and homogenised in ice-cold Tris/NaCl buffer, at a pH of 7.0. Samples were then centrifuged at 15000 rpm for 10 min at 4 °C and supernatant was collected for further analysis. Total protein quantification was carried out as described by Bradford (1976), adapted to a 96-wells microplate. Protein Assay Dye Reagent Concentrate (Biorad ®) was diluted in ultra-pure water at a concentration of 1:4. Samples were distributed throughout microplates and absorbance was read at 600 nm. Protein quantification was carried out using a Thermo Scientific Multiskan ® EX Microplate reader (Thermo Scientific, Waltham, MA, USA). Total protein content was obtained through calibration curves created by comparison with different concentrations of the bovine gamma globulin standard.

#### 2.4.3. Carbohydrate analysis

Carbohydrate analysis of bivalve tissue comprised the quantification of polysaccharide and monosaccharide content, namely neutral sugars and total uronic acids. For polysaccharide analysis, samples were subjected to hydrolysis followed by reduction and acetylation, as described in Coimbra et al. (1996). Neutral sugars from monosaccharide analysis were not subjected to hydrolysis but followed the same protocol for reduction and acetylation. The alditol acetate derivates obtained in the polysaccharide and monosacharide analyses were separated in a Clarus 400 Gas Chromatography equipment (PerkinElmer ®, Krakow, Poland) associated to a Flame Ionization Detector (GC-FID). A DB-225 capillary column (30 m length × 0.25 mm i.d. × 0.15 μm film thickness; J&W Scientific, Folsom, CA, USA) was used. 2 μL of samples, dissolved in anhydrous acetone, were injected per run. Each run had a 11 min duration. The injection temperature was 220 °C and the oven temperature was set to increase from 200 °C to 220 °C at a 40 °C $min^{-1}$ rate, stabilize at 220 °C for 7 min, and increase to 230 °C at 20 °C $min^{-1}$ rate, finally maintaining this temperature for 1 min. The carrier gas was $H_2$, at a flow rate of 1.7 mL $min^{-1}$. Quantification of sugars was obtained by comparison of the sugar chromatographic peak areas to the peak areas obtained for the standard used (2-desoxiglucose). Total uronic acid content was measured by a colorimetric procedure described in Selvendran et al. (1979) and Coimbra et al. (1996). Uronic acid aliquots were obtained during the polysaccharide hydrolysis and m-phenylphenol (MPP) was the dye reagent used. Samples absorbance was read at 520 nm, using a BioTek™ Eon Microplate Spectrophotometer (Winooski, VT, USA). Total uronic acid content was obtained through calibration curves created with different concentrations of the galacturonic acid standard.

### 2.5. Fatty acid trophic markers

Fatty Acid Trophic Markers (FATMs) present in the bivalves' tissues were calculated, based on Prato et al. (2010) and Ezgeta-Balić et al. (2012), to determine the food preferences of each bivalve species in both seasons and geographical locations. PUFAs are associated with a diet rich in phytoplankton whereas SFAs are associated with a consumption of detritus (Volkman et al., 1989; Fahl and Kattner, 1993). High quantities of DHA in bivalves are associated with a consumption of dinoflagellates while EPA is related to a consumption of diatoms (Budge and Parrish, 1998). C16:1n-7 t is characteristic of a diet based on diatoms, while C16:0 is related to dinoflagellate consumption (Graeve et al., 1994a, 1994b). C18:1n-9, C18:2n-6, C20:1n-9 and DHA are fatty acids found in higher contents in bivalves that feed on zooplankton (Virtue et al., 2000; Kharlamenko et al., 2001). The sum of branched fatty acids (iso and ante-iso branched chains) C15:0 and C17:0 is used to determine the bacterial and detritus consumption (Mayzaud et al., 1989; Nadjek et al., 2002).

### 2.6. Statistical analysis

Multivariate statistical analysis was carried out with the PRIMER-6 software to examine the fatty acid, polysaccharide, monosaccharide and FATMs profiles for discriminatory information about spatial and seasonal variations (Clarke and Gorley, 2006). Non-metric multidimensional scaling (n-MDS) plots were conducted to address the variations and the groups formed according to the bivalves' biochemical composition. Biochemical data was converted into similarity matrices, using a Bray-Curtis coefficient, and tested with a one-way analysis of similarity (ANOSIM), taking into consideration the species, studied areas and season. Each biochemical component and FATMs influences

**Table 2**
Abundance of fatty acids (μg/g) of the bivalve species sampled in the Mondego estuary (M) and in the Ria Formosa lagoon (RF), in the winter of 2016 (W) and in the summer of 2017 (S).

| Species | | *Cerastoderma edule* | | | | *Crassostrea gigas* | | *Mytilus galloprovincialis S* | | *Mytilus galloprovincialis B* | | | | *Ruditapes decussatus* | | | | *Scrobicular ia plana* | | *Solen marginatus* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Area | | M | | RF | | RF | | M | | M | RF | M | RF | M | RF | M | RF | M | | M | RF | M | RF |
| Season | | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S |
| Fatty Acids | C13:0 | 0.01 | 0.00 | 0.03 | | 0.00 | | 1.64 | 0.04 | 0.01 | 0.00 | | 0.01 | 0.02 | | 0.00 | 0.00 | | | 0.01 | | | |
| | C14:0 | 0.02 | 0.01 | 0.92 | 0.47 | 0.00 | 0.00 | 3.87 | 0.51 | 0.09 | 0.03 | 0.04 | 0.01 | 0.24 | 0.34 | 0.03 | 0.00 | 0.18 | 0.02 | 0.00 | 0.00 | 0.01 | 0.00 |
| | C15:0 | 0.03 | 0.01 | 0.26 | 0.06 | 0.00 | 0.00 | 1.22 | 0.26 | 0.06 | 0.02 | 0.05 | 0.01 | 0.20 | 0.15 | 0.11 | 0.01 | 0.14 | 0.01 | 0.02 | 0.00 | 0.03 | 0.00 |
| | C16:0 | 0.14 | 0.07 | 4.33 | 1.46 | 0.06 | 0.01 | 29.19 | 4.53 | 1.38 | 0.26 | 0.34 | 0.12 | 4.47 | 4.85 | 0.10 | 0.09 | 1.59 | 0.07 | 0.03 | 0.01 | 0.03 | 0.01 |
| | C17:0 | 0.07 | 0.04 | 0.51 | 0.27 | 0.01 | 0.01 | 2.18 | 0.36 | 0.14 | 0.06 | 0.03 | 0.11 | 0.46 | 0.55 | 0.22 | 0.07 | 0.57 | 0.02 | 0.05 | 0.01 | 0.01 | 0.01 |
| | C18:0 | 0.06 | 0.04 | 2.20 | 0.57 | 0.01 | 0.00 | 7.20 | 1.24 | 0.28 | 0.05 | 0.13 | 0.05 | 2.02 | 1.23 | 0.02 | 0.03 | 0.88 | 0.03 | 0.01 | 0.00 | 0.01 | 0.01 |
| | C20:0 | 0.02 | 0.05 | 0.78 | 0.38 | | | | | | | 0.03 | 0.03 | | 0.86 | | | 2.63 | | | | | |
| | C21:0 | 0.02 | | | | 0.01 | | 3.02 | | 0.02 | 0.05 | 0.01 | | 0.13 | | 0.02 | | 0.68 | | 0.01 | | 0.00 | |
| | C22:0 | 0.04 | 0.02 | 1.09 | 0.65 | 0.00 | | 1.45 | 0.64 | 0.21 | 0.02 | 0.04 | 0.01 | 0.54 | 0.71 | 0.01 | 0.01 | 0.15 | | | | | |
| | C23:0 | 0.01 | 0.01 | 0.67 | 0.26 | | | 1.97 | 0.56 | 0.09 | | 0.04 | 0.01 | 0.51 | 0.35 | | 0.00 | 0.18 | 0.01 | 0.01 | | | |
| | C24:0 | | | | | | | | | | | | 0.01 | 0.46 | | | | | | | | | |
| | **Total SFA** | 0.42 | 0.25 | 10.79 | 4.12 | 0.11 | 0.02 | 51.74 | 8.14 | 2.28 | 0.49 | 0.71 | 0.37 | 9.06 | 9.04 | 0.51 | 0.21 | 7.00 | 0.16 | 0.15 | 0.02 | 0.09 | 0.03 |
| | C14:1n-5t | 0.01 | 0.00 | 0.21 | 0.12 | 0.00 | 0.00 | 0.28 | 1.07 | 0.05 | 0.01 | 0.02 | 0.00 | 0.06 | 0.14 | 0.01 | 0.00 | 0.10 | 0.01 | 0.01 | 0.00 | 0.01 | 0.00 |
| | C15:1n-5c | 0.04 | 0.01 | 1.05 | 0.37 | 0.03 | 0.00 | 1.24 | 0.10 | 0.06 | 0.14 | 0.12 | 0.04 | 0.21 | 0.28 | 0.05 | 0.03 | 0.14 | 0.01 | 0.01 | 0.00 | 0.03 | 0.00 |
| | C16:1n-7t | 0.14 | 0.07 | 0.58 | 0.66 | 0.02 | 0.01 | 2.03 | 3.69 | 0.08 | 0.05 | 0.09 | 0.07 | 1.13 | 1.20 | 0.04 | 0.06 | 0.45 | 0.04 | 0.07 | 0.01 | 0.01 | 0.01 |
| | C17:1n-8c | 0.18 | 0.07 | 0.66 | 0.51 | 0.22 | 0.04 | 3.87 | 0.65 | 0.76 | 0.08 | 0.36 | 0.45 | 1.09 | 1.01 | 0.01 | 0.22 | 0.48 | 0.04 | 0.15 | 0.02 | 0.06 | 0.02 |
| | C18:1n-9t | 0.01 | 0.01 | 0.51 | 0.31 | 0.01 | 0.00 | 3.18 | 0.98 | 0.13 | 0.04 | 0.04 | 0.02 | 1.07 | 0.77 | 0.01 | 0.01 | 1.06 | 0.02 | 0.01 | 0.00 | 0.00 | 0.00 |
| | C18:1n-9c | 0.02 | 0.02 | 0.50 | 0.15 | 0.02 | 0.00 | 3.26 | | 0.12 | | 0.05 | 0.01 | 0.48 | 0.18 | | 0.00 | 0.48 | 0.01 | 0.01 | 0.00 | 0.01 | 0.00 |
| | C20:1n-9c | 0.02 | 0.01 | 0.63 | 0.32 | 0.02 | | 24.02 | 5.63 | 0.48 | 0.08 | 0.19 | 0.04 | 0.56 | 0.67 | | | 0.52 | 0.06 | 0.00 | | 0.01 | 0.00 |
| | C22:1n-9c | 0.03 | 0.02 | 0.87 | 0.49 | 0.01 | | 9.35 | 2.60 | 0.31 | 0.05 | 0.18 | 0.09 | 1.54 | 1.53 | 0.02 | 0.04 | 0.05 | 0.00 | | | 0.01 | |
| | **Total MUFA** | 0.45 | 0.21 | 5.01 | 2.93 | 0.34 | 0.06 | 47.23 | 14.72 | 1.99 | 0.45 | 1.05 | 0.72 | 6.14 | 5.78 | 0.14 | 0.36 | 3.28 | 0.19 | 0.26 | 0.03 | 0.14 | 0.03 |
| | C18:2n-6t | | 0.00 | | | 0.01 | 0.00 | | 1.80 | | | | | | | | 0.01 | | | | | | 0.00 |
| | C18:2n-6c (LA) | 0.01 | 0.01 | 0.41 | 0.08 | | | 3.27 | | 0.14 | 0.05 | 0.03 | 0.02 | 0.19 | 0.17 | | 0.01 | 0.20 | 0.01 | 0.00 | 0.00 | 0.01 | 0.00 |
| | C18:3n-6t | | | | 0.08 | | | | | | | | | | | | | 0.22 | | | 0.00 | | 0.00 |
| | C18:3n-3c (ALA) | 0.01 | 0.02 | 0.68 | 0.17 | 0.02 | | 1.53 | 1.38 | 0.11 | 0.04 | 0.02 | 0.01 | 0.30 | 0.39 | | 0.00 | 0.21 | 0.02 | 0.01 | 0.00 | 0.01 | 0.01 |
| | C20:2n-6c | 0.02 | 0.02 | 0.50 | 0.19 | 0.02 | | 1.73 | 1.67 | 0.65 | 0.12 | 0.30 | 0.02 | | 0.78 | | 0.01 | | | | | | |
| | C22:2n-6c | 0.06 | 0.02 | 2.09 | 0.58 | 0.09 | | 3.39 | 1.51 | 0.21 | 0.03 | 0.06 | 0.03 | 0.43 | 0.43 | 0.01 | 0.00 | 0.13 | 0.00 | 0.01 | 0.01 | | |
| | **Total PUFA** | 0.10 | 0.07 | 3.68 | 1.10 | 0.14 | 0.00 | 9.92 | 6.36 | 1.11 | 0.24 | 0.41 | 0.08 | 0.92 | 1.77 | 0.01 | 0.03 | 0.76 | 0.03 | 0.02 | 0.01 | 0.02 | 0.01 |
| | C20:3n-7c | 0.02 | | | | | | | 0.73 | 0.07 | | 0.01 | | 0.87 | 0.13 | | | | | | 0.01 | | 0.01 |
| | C20:4n-6c (ARA) | 0.04 | 0.04 | 2.25 | 1.25 | 0.02 | 0.02 | 10.00 | 2.37 | 0.47 | | 0.43 | 0.14 | 1.13 | 1.21 | | 0.03 | 1.01 | 0.03 | | | 0.02 | |
| | C20:5n-3c (EPA) | 0.19 | 0.12 | 6.00 | 2.43 | 0.10 | 0.00 | 21.58 | 8.48 | 1.77 | 0.29 | 0.50 | 0.14 | 2.36 | 4.14 | 0.02 | 0.03 | 0.99 | 0.13 | 0.03 | | 0.05 | |
| | C22:6n-6c (DHA) | 0.32 | 0.20 | 12.80 | 4.83 | 0.19 | 0.01 | 73.56 | 14.95 | 3.98 | 0.57 | 1.08 | 0.40 | 14.13 | 13.75 | 0.11 | 0.08 | 4.61 | 0.14 | 0.07 | 0.01 | 0.09 | 0.02 |
| | **Total HUFA** | 0.57 | 0.36 | 21.05 | 8.51 | 0.31 | 0.03 | 105.14 | 26.53 | 6.29 | 0.86 | 2.02 | 0.68 | 18.49 | 19.23 | 0.13 | 0.14 | 6.61 | 0.30 | 0.10 | 0.02 | 0.16 | 0.03 |
| | **Total FA** | 1.54 | 0.89 | 40.53 | 16.67 | 0.90 | 0.11 | 214.03 | 55.75 | 11.67 | 2.04 | 4.19 | 1.85 | 34.61 | 35.82 | 0.79 | 0.74 | 17.65 | 0.68 | 0.53 | 0.08 | 0.41 | 0.10 |
| | **N** | 22 | 21 | 20 | 20 | 21 | 11 | 20 | 19 | 21 | 17 | 21 | 21 | 22 | 20 | 13 | 19 | 20 | 16 | 17 | 13 | 15 | 15 |

the similarities and dissimilarities within and between sample groups. Similarities and dissimilarities were verified through a similarity percentage analysis routine (SIMPER) (Clarke and Warwick, 1994). Total uronic acid content values were included in the polysaccharide statistical analysis, as they were considered sugar residues of polysaccharides. Total protein content had non-normal distribution of data and no homogeneity of variances. Non-parametric tests were applied in this case and were analysed with the STATISTICA-7 software (StatSoft Inc, 2004). Samples of protein content were divided in separate groups, considering the bivalve species, where and when they were sampled. 22 groups were formed with 6 replicates in each group. To estimate significant differences between group distributions of total protein content from different bivalve species in both seasons and geographical locations, a Kruskal-Wallis H test was made, followed by a series of Mann-Whitney U tests to estimate which groups had significant different distributions ($p \leq 0.05$).

## 3. Results

### 3.1. Fatty acid composition

FA profiles were described in terms of biochemical abundance for each bivalve species sampled in the Mondego estuary and in the Ria Formosa lagoon, in winter and summer seasons (Table 2). Several fatty acids remained abundant among all bivalve species. The most abundant SFAs were C16:0, C17:0 and C18:0, while C16:1n-7 cis, C17:1n-8 cis, C20:1n-9 cis and C22:1n-9 cis were the most abundant MUFAs. All species were rich in PUFAs, particularly in HUFAs such as ARA, EPA and DHA. ALA and LA were other fatty acids that are at the FA composition of the studied bivalves, but in lower abundance. Spatial variations in FA profiles were detected in *C. edule*, exhibiting higher abundance in the lagoon system in both seasons; in *M. galloprovincialis* B, presenting higher abundance in the Mondego estuary in winter; in *R. decussatus*, showing higher abundance in the Mondego estuary in both seasons. Seasonal variations in FA profiles were observed in *C. edule* from both study areas, in *M. galloprovincialis* S, *M. galloprovincialis* B, *S. plana* and *S. marginatus* from the Mondego estuary, in *C. gigas* and *R. decussatus* from the Ria Formosa lagoon, with higher FA abundance in winter. When comparing the different sizes of *M. galloprovincialis* sampled in the Mondego estuary, the small organisms exhibited higher FA content than big organisms. *M. galloprovincialis* S and *C. edule* presented the highest FA profile in both seasons in the Mondego estuary and in the Ria Formosa lagoon, respectively.

The two-dimensional n-MDS plot (Fig. 2) showed a separation of samples based on FA abundance and composition (stress = 0.02). Four groups were defined. Group A contained the bivalve species that had the less diversified and the lowest abundance in FA. Group B comprised the species that had a significant higher abundance on FA than group A. Group C was formed by the species that had a significant higher abundance in FA than the previous groups. Group D included the species that presented the highest abundance in FA from all groups formed. ANOSIM analysis indicated a clear separation of the groups defined ($R = 0.91$; $p = 0.00$). When comparing pairwise tests, almost all groups were significantly different ($p \leq 0.05$) and presented high R values, showing good segregation (A/B: $R = 0.94$, $p = 0.00$; A/D: $R = 1$, $p = 0.03$; B/C: $R = 0.93$, $p = 0.00$; B/D: $R = 0.99$, $p = 0.00$; C/D: $R = 1$, $p = 0.03$). Groups A and C ($R = 1$, $p = 0.1$) had strong segregation, but were not significantly different. SIMPER analysis (Table 3) showed that the average similarities were explained predominantly by C17:1n-8c, DHA, C16:0, C17:0 and C16:1n-7t in group A; DHA, C17:1n-8c, EPA, C16:0 and C16:1n-7t in group B; DHA, C16:0, EPA, ARA and C17:1n-8c in group C; DHA, C16:0, EPA, C18:0 and ARA in group D. In what concerns the dissimilarities between groups, the main contributors were DHA, C17:1n-8c, EPA, C16:0 and C16:1n-7t among A/B groups; DHA, EPA, C16:0, C20:0 and ARA among groups A/C and B/C; DHA, EPA, C16:0, C18:0 and ARA among groups A/D and B/D; DHA, EPA, C16:0, C22:1n-9c and C20:1n-9c among groups C/D.

### 3.2. Total protein content

Kruskal-Wallis H test ($H = 93.76$, $p < 0.05$) showed significant differences between the groups' distributions considering the total protein content of different bivalve species in both seasons and study areas. Based on the pairwise two-tailed Mann-Whitney U tests performed (Fig. 3), seasonal variations of protein content were detected in *C. edule* and *M. galloprovincialis* B, from both study areas, and in *C. gigas*, *R. decussatus* and *S. marginatus*, from the lagoon system, with significantly higher contents in winter ($2.24 < Z < 2.82$; $N = 12$; $0.00 < p < 0.03$). *M. galloprovincialis* S, *R. decussatus*, *S. plana* and *S. marginatus* collected in the Mondego estuary presented non-significantly higher protein contents in winter than in summer ($0.64 < Z < 1.12$; $N = 12$; $0.31 < p < 0.59$). The size variation did not have an impact in the total protein content of *M. galloprovincialis*, from the Mondego estuary, since, in summer, both sizes showed similar protein content and, in winter, big

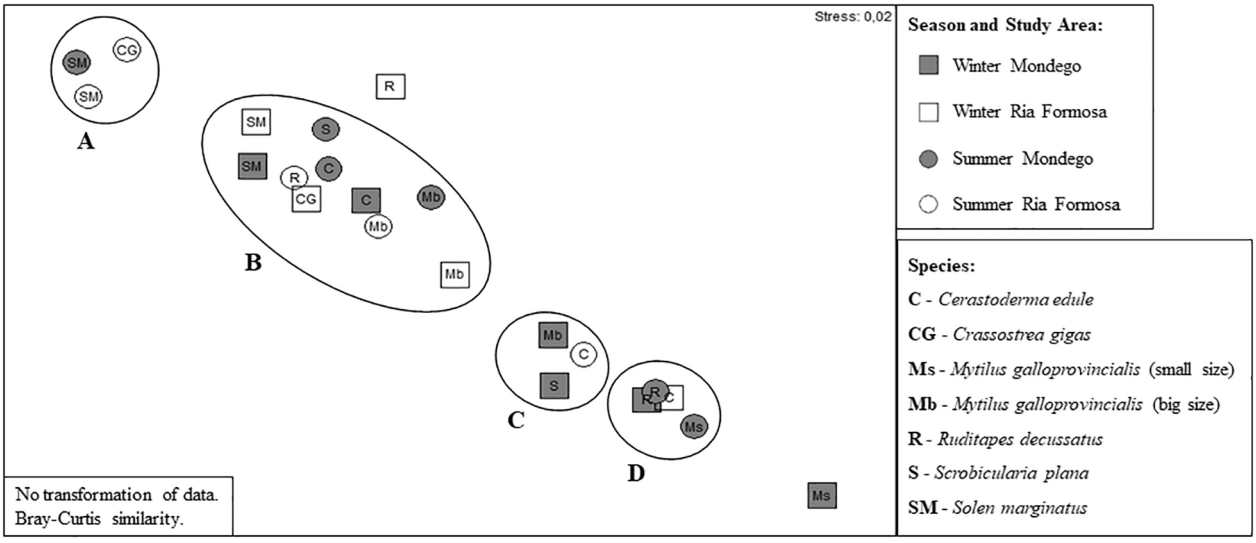


**Fig. 2.** Two-dimensional n-MDS plot of fatty acid composition of the bivalve species sampled at the Mondego estuary and at the Ria Formosa lagoon, in winter and summer seasons. A, B, C and D were the groups defined in the n-MDS.

**Table 3**
SIMPER analysis of fatty acid abundance showing average similarities and dissimilarities between the species from each group defined in the n-MDS plot.

| Group | Average Similarity | Fatty Acids | Av. Abund | | Av. Sim | Sim/SD | Contrib % | Cum. % |
|---|---|---|---|---|---|---|---|---|
| A | 66.72 | C17:1n-8c | 0.03 | | 21.80 | 9.81 | 32.67 | 32.67 |
| | | C22:6n-6c DHA | 0.02 | | 11.71 | 9.19 | 17.56 | 50.23 |
| | | C16:0 | 0.01 | | 7.49 | 6.20 | 11.23 | 61.46 |
| | | C17:0 | 0.01 | | 6.18 | 7.74 | 9.26 | 70.71 |
| | | C16:1n-7t | 0.01 | | 5.03 | 6.14 | 7.54 | 78.26 |
| B | 51.52 | C22:6n-6c DHA | 0.31 | | 11.65 | 2.45 | 22.61 | 22.61 |
| | | C17:1n-8c | 0.18 | | 8.70 | 1.50 | 16.88 | 39.49 |
| | | C20:5n-3c EPA | 0.16 | | 6.10 | 1.85 | 11.85 | 51.34 |
| | | C16:0 | 0.12 | | 4.96 | 2.44 | 9.62 | 60.96 |
| | | C16:1n-7t | 0.06 | | 3.47 | 1.59 | 6.73 | 67.69 |
| C | 70.58 | C22:6n-6c DHA | 4.47 | | 27.34 | 44.06 | 38.74 | 38.74 |
| | | C16:0 | 1.48 | | 9.21 | 14.75 | 13.05 | 51.80 |
| | | C20:5n-3c EPA | 1.73 | | 8.35 | 2.30 | 11.83 | 63.63 |
| | | C20:4n-6c ARA | 0.91 | | 4.14 | 2.76 | 5.86 | 69.49 |
| | | C17:1n-8c | 0.58 | | 3.22 | 8.13 | 4.57 | 74.06 |
| D | 77.82 | C22:6n-6c DHA | 13.91 | | 32.42 | 7.69 | 41.66 | 41.66 |
| | | C16:0 | 4.54 | | 10.72 | 7.89 | 13.78 | 55.44 |
| | | C20:5n-3c EPA | 5.24 | | 8.42 | 2.97 | 10.83 | 66.27 |
| | | C18:0 | 1.67 | | 3.36 | 3.21 | 4.31 | 70.58 |
| | | C20:4n-6c ARA | 1.74 | | 3.20 | 4.14 | 4.12 | 74.70 |
| Groups | Average Dissimilarity | Fatty Acids | Av. Abund | | Av. Diss | Diss/SD | Contrib % | Cum. % |
| A/B | 82.28 | C22:6n-6c DHA | 0.02 | 0.31 | 17.35 | 3.09 | 21.09 | 21.09 |
| | | C17:1n-8c | 0.03 | 0.18 | 11.56 | 1.42 | 14.05 | 35.14 |
| | | C20:5n-3c EPA | 0.00 | 0.16 | 9.89 | 2.50 | 12.02 | 47.15 |
| | | C16:0 | 0.01 | 0.12 | 6.84 | 2.79 | 8.32 | 55.47 |
| | | C16:1n-7t | 0.01 | 0.06 | 4.65 | 1.55 | 5.65 | 61.12 |
| A/C | 98.73 | C22:6n-6c DHA | 0.02 | 4.47 | 29.40 | 8.71 | 29.77 | 29.77 |
| | | C20:5n-3c EPA | 0.00 | 1.73 | 11.70 | 2.55 | 11.85 | 41.63 |
| | | C16:0 | 0.01 | 1.48 | 9.74 | 6.87 | 9.86 | 51.49 |
| | | C20:0 | 0.00 | 1.00 | 5.70 | 0.83 | 5.77 | 57.26 |
| | | C20:4n-6c ARA | 0.01 | 0.91 | 5.67 | 3.78 | 5.74 | 63.00 |
| A/D | 99.53 | C22:6n-6c DHA | 0.02 | 13.91 | 34.28 | 5.96 | 34.44 | 34.44 |
| | | C20:5n-3c EPA | 0.00 | 5.24 | 12.06 | 3.44 | 12.12 | 46.56 |
| | | C16:0 | 0.01 | 4.54 | 11.26 | 5.12 | 11.31 | 57.87 |
| | | C18:0 | 0.00 | 1.67 | 4.21 | 2.76 | 4.23 | 62.11 |
| | | C20:4n-6c ARA | 0.01 | 1.74 | 4.09 | 4.29 | 4.11 | 66.21 |
| B/C | 84.37 | C22:6n-6c DHA | 0.31 | 4.47 | 25.43 | 6.26 | 30.14 | 30.14 |
| | | C20:5n-3c EPA | 0.16 | 1.73 | 9.88 | 2.37 | 11.71 | 41.84 |
| | | C16:0 | 0.12 | 1.48 | 8.34 | 5.59 | 9.89 | 51.73 |
| | | C20:0 | 0.01 | 1.00 | 5.31 | 0.86 | 6.30 | 58.03 |
| | | C20:4n-6c ARA | 0.07 | 0.91 | 4.88 | 2.85 | 5.78 | 63.81 |
| B/D | 93.71 | C22:6n-6c DHA | 0.31 | 13.91 | 32.52 | 6.05 | 34.71 | 34.71 |
| | | C20:5n-3c EPA | 0.16 | 5.24 | 11.34 | 3.30 | 12.11 | 46.81 |
| | | C16:0 | 0.12 | 4.54 | 10.65 | 5.21 | 11.37 | 58.18 |
| | | C18:0 | 0.04 | 1.67 | 3.99 | 2.80 | 4.26 | 62.44 |
| | | C20:4n-6c ARA | 0.07 | 1.74 | 3.81 | 3.85 | 4.06 | 66.50 |
| C/D | 51.03 | C22:6n-6c DHA | 4.47 | 13.91 | 16.85 | 6.43 | 33.03 | 33.03 |
| | | C20:5n-3c EPA | 1.73 | 5.24 | 5.80 | 1.71 | 11.36 | 44.38 |
| | | C16:0 | 1.48 | 4.54 | 5.51 | 5.42 | 10.80 | 55.19 |
| | | C22:1n-9c | 0.29 | 1.63 | 2.31 | 2.58 | 4.53 | 59.72 |
| | | C20:1n-9c | 0.44 | 1.87 | 2.08 | 0.66 | 4.08 | 63.80 |

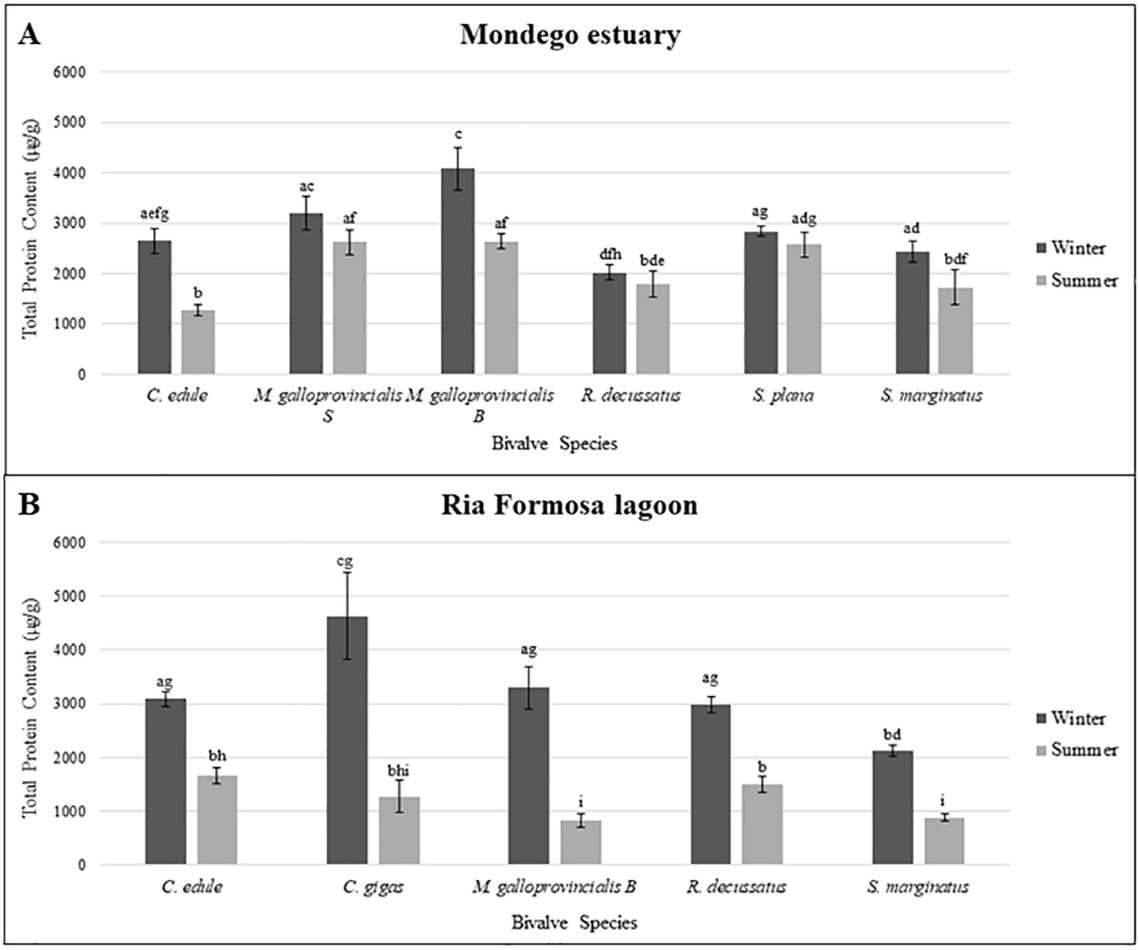


**Fig. 3.** Total protein content of the bivalve species sampled at the Mondego estuary (A) and at the Ria Formosa lagoon (B), in winter 2016 (dark grey) and summer 2017 (light grey) seasons. Mean and standard error are shown in the data bars and error bars, respectively. The letters on the top of the bars stand for similar protein content ($p > 0.05$). Different letters represent statistical differences ($p \leq 0.05$) between protein content within each species, season and study area (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

size organisms revealed a non-significant higher protein content ($0.64 < Z < 1.12$, $N = 12$, $0.31 < p < 0.59$). Spatial variations of protein content were observed in *M. galloprovincialis* B and *S. marginatus* from summer, with the estuarine samples presenting higher protein content, and in *R. decussatus* from winter, demonstrating higher content in the Ria Formosa lagoon ($2.24 < Z < 2.88$; $N = 12$; $0.00 < p < 0.03$). In the Mondego estuary, *M. galloprovincialis* exhibited the highest protein content in both seasons (size S: 3197.62 ± 332.37 µg/g in winter and 2634.11 ± 248.43 µg/g in summer; size B: 4076.92 ± 420.08 µg/g in winter and 2634.05 ± 358.42 µg/g in summer). In the lagoon system, *C. gigas* and *C. edule* showed the highest protein content in winter (4626.82 ± 813.83 µg/g) and in summer (1660.41 ± 156.55 µg/g), respectively.

### 3.3. Carbohydrate composition

Polysaccharide composition of bivalves was described in terms of monosaccharide residues for the bivalve species harvested in the Mondego estuary and in the Ria Formosa lagoon, in winter and in summer (Table 4). In both study areas and seasons, the most abundant residue was glucose. In much lower concentrations were detected xylose, rhamnose, fucose, ribose, arabinose, mannose, galactose and uronic acids. Based on the results of the multivariate statistical analysis, seasonal variations of polysaccharide abundance were observed in *C. edule*, *M. galloprovincialis* S, *S. plana* and *S. marginatus* from the Mondego estuary, with higher abundance in winter; and in *C. edule*, *R. decussatus* and *S. marginatus* sampled in the lagoon system, with higher abundance in summer. Spatial variations were observed in *M. galloprovincialis* B in both seasons and *R. decussatus* and *S. marginatus* in winter, demonstrating higher polysaccharide abundance in the Mondego estuary than in the Ria Formosa lagoon. *M. galloprovincialis* S from the Mondego estuary showed higher abundance of polysaccharides in winter and lower in summer, than the big size organisms. The highest polysaccharide compositions were observed in *R. decussatus*, sampled in winter

**Table 4**
Abundance of sugar residues (μg/g) in polysaccharides of the bivalve species sampled in the Mondego estuary (M) and in the Ria Formosa lagoon (RF), in the winter of 2016 (W) and in the summer of 2017 (S).

| Species | | *Cerastoderma edule* | | | | *Crassostrea gigas* | | *Mytilus galloprovincialis* S | | *Mytilus galloprovincialis* B | | | | *Ruditapes decussatus* | | | | *Scrobicularia plana* | | *Solen marginatus* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Area | | M | | RF | | RF | | M | | M | | RF | | M | | RF | | M | | M | | RF | |
| Season | | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S |
| Sugar residues | Rhamnose | 13.55 | 7.81 | 7.19 | 43.35 | 2.06 | 2.97 | | 43.92 | | 13.85 | | 4.23 | 84.41 | 21.22 | | 19.33 | 106.80 | 21.45 | | 11.25 | 1.23 | 8.29 |
| | Fucose | 39.10 | 13.57 | 87.08 | 141.90 | 3.84 | 5.43 | 741.02 | 119.67 | 125.05 | 21.28 | 39.16 | 25.46 | 218.27 | 163.10 | 19.58 | 74.70 | 119.32 | 4.25 | | 11.17 | 4.83 | 9.50 |
| | Ribose | 28.45 | 35.43 | 115.84 | 287.16 | 8.55 | 12.63 | 768.39 | 141.67 | 199.36 | 45.85 | 24.42 | 39.02 | 76.95 | 184.05 | 14.21 | 146.20 | 99.64 | 16.45 | 45.13 | 79.04 | 19.24 | 61.49 |
| | Arabinose | 23.94 | 9.25 | 14.76 | 50.72 | 0.94 | 4.75 | | 15.62 | 24.89 | 10.06 | | 3.63 | 61.38 | 13.73 | | 12.63 | 77.37 | 9.16 | 12.60 | 9.27 | 2.15 | 6.58 |
| | Xylose | 179.26 | 10.84 | 114.00 | 196.83 | 27.50 | 16.58 | 337.14 | 144.93 | 105.74 | 121.22 | 110.90 | | 266.33 | 162.73 | 15.56 | 216.80 | 299.12 | 35.32 | 186.89 | 27.48 | 9.10 | 37.59 |
| | Mannose | 16.43 | 10.27 | 41.86 | 75.39 | 2.94 | 23.85 | 569.94 | 49.24 | 38.51 | 10.74 | 7.58 | 10.96 | 91.38 | 28.92 | 11.98 | 51.02 | 125.91 | 9.76 | 14.53 | 18.51 | 7.34 | 17.33 |
| | Galactose | 24.93 | 20.15 | 63.48 | 189.07 | 5.58 | 7.76 | 927.32 | 90.74 | 104.50 | 20.78 | 41.61 | 25.00 | 154.54 | 205.38 | 20.11 | 94.48 | 149.59 | 6.37 | 28.38 | 23.71 | 10.40 | 18.80 |
| | Glucose | 3718.70 | 349.17 | 1132.63 | 6481.07 | 752.87 | 571.96 | 3501.27 | 2012.65 | 5000.52 | 3192.29 | 218.19 | 176.06 | 9593.63 | 2656.73 | 687.20 | 2396.63 | 7760.92 | 1087.60 | 3991.25 | 1455.55 | 334.46 | 1424.75 |
| | Uronic Acids | 14.68 | 35.76 | 85.65 | 31.93 | 29.01 | 9.15 | 690.21 | 157.65 | 109.63 | 82.79 | 13.95 | 20.11 | 60.20 | 37.26 | 29.17 | 14.87 | 55.34 | 190.14 | 44.59 | 10.04 | 19.63 | 7.36 |
| | **Total FA** | 4059.04 | 492.25 | 1662.49 | 7497.42 | 833.29 | 655.08 | 7535.29 | 2776.09 | 5708.20 | 3518.86 | 455.81 | 304.47 | 10607.09 | 3473.12 | 797.81 | 3026.66 | 8794.01 | 1380.50 | 4323.37 | 1646.02 | 408.38 | 1591.69 |
| | **N** | 9 | 9 | 9 | 9 | 9 | 9 | 7 | 9 | 8 | 9 | 7 | 8 | 9 | 9 | 7 | 9 | 9 | 9 | 7 | 9 | 9 | 9 |

(10607.09 μg/g), and in *M. galloprovincialis* B, collected in summer (3518.86 μg/g), in the Mondego estuary, and in *C. edule*, sampled in both seasons (1662.49 μg/g in winter and 7497.42 μg/g in summer), in the Ria Formosa lagoon.

The two-dimensional n-MDS plot (Fig. 4) shows an apparent distribution of the samples according to the study areas and the abundance of sugar residues in polysaccharides (stress = 0.02). Three groups were defined. Group A included the species that presented highest abundance in sugar residues, where most of them sampled in the Mondego estuary. Group B comprised the species that had a significant lower abundance of sugarresidues than group A. Group C comprised almost all species from the Ria Formosa lagoon that showed lower abundance of polysaccharides. ANOSIM analysis indicated a clear segregation of the three groups defined ($R = 0.83$; $p = 0.00$). When comparing pairwise tests, all groups were significantly different ($p \leq 0.05$) and presented high R values, showing good segregation between each other (A/B: $R = 0.71$, $p = 0.00$; A/C: $R = 0.99$, $p = 0.00$; B/C: $R = 0.66$, $p = 0.01$). SIMPER analysis (Table 5) showed that average similarities were explained by glucose and xylose in group A; glucose and ribose in group B; glucose, uronic acids, ribose and galactose in group C. Average dissimilarities were explained by glucose, xylose and ribose between groups A/B; glucose and xylose between groups A/C; glucose, uronic acids, ribose and xylose between groups B/C.

The monosaccharide profile was described in terms of neutral sugars for the bivalve species harvested at the Mondego estuary and at the Ria Formosa lagoon, in winter and summer (Table 6). In both study areas and seasons, the most abundant neutral sugar was glucose. Xylose and fucose were the second most present neutral sugars in the samples from the Mondego estuary and the Ria Formosa lagoon, respectively. The other neutral sugars detected were rhamnose, ribose, arabinose, mannose and galactose. Based on the results of the multivariate statistical analysis, seasonal variations were detected in *C. edule*, that showed higher monosaccharide abundance in summer, at the Mondego estuary, and in winter, at the Ria Formosa lagoon; in *C. gigas* and *S. marginatus*, that exhibited higher abundance in winter in the lagoon system; in *M. galloprovincialis* S and *S. marginatus*, presenting higher abundance in summer in the estuarine system; *M. galloprovincialis* B, from both study areas, demonstrating higher abundance in summer. Spatial variations were observed in winter, with *C. edule* presenting higher monosacharide abundance in the Ria Formosa lagoon, and in both seasons, with *M. galloprovincialis* B and *R. decussatus* demonstrating higher monosaccharide abundance in the Mondego estuary and in the Ria Formosa lagoon, respectively. Size variation of monosaccharide abundance was observed in the estuarine system, with *M. galloprovincialis* S showing higher abundance in both seasons, than big size organisms. The highest monosaccharide abundances were detected in *M. galloprovincialis* S (468.68 μg/g in winter and 919.14 μg/g in summer) and in *C. edule* (1473.07 μg/g in winter and 349.58 μg/g in summer), in both seasons, sampled in the Mondego estuary and in the Ria Formosa lagoon, respectively.

The two-dimensional n-MDS analysis (Fig. 5) showed a separation of the samples based on monosaccharide abundance (stress = 0.03). Three groups were defined. Group A comprised the bivalve species that showed the lowest monosaccharide abundance. Group B was composed by species that presented a significant lower abundance of monosaccharides than the species from group C. Group C contained the species that exhibited the highest monosaccharide abundance. ANOSIM analysis indicated a clear segregation of the three groups defined ($R = 0.79$; $p = 0.00$). When comparing pairwise tests, all groups were significantly different ($p \leq 0.05$) and presented high R values, showing good segregation (A/B: $R = 0.79$, $p = 0.00$; A/C: $R = 1$, $p = 0.01$; B/C: $R = 0.82$, $p = 0.00$). SIMPER analysis (Table 7) showed that average similarities were explained by glucose in groups A and B; by glucose and arabinose in group C. Average dissimilarities were explained by glucose, ribose, fucose and arabinose between groups A/B; glucose followed by arabinose and xylose among groups A/C; glucose, xylose, arabinose and

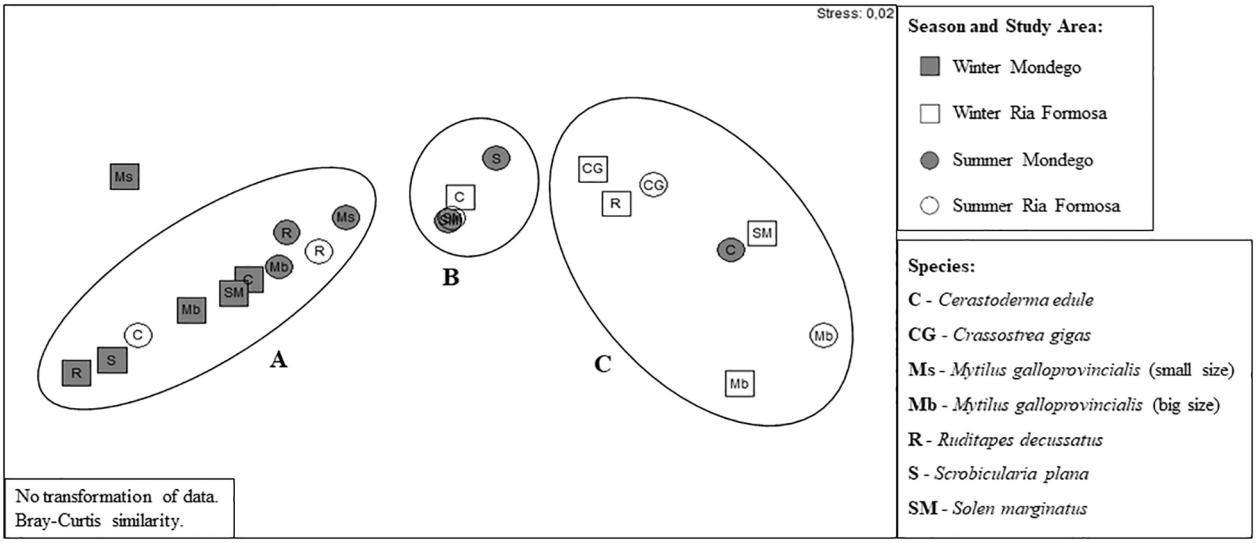


**Fig. 4.** Two-dimensional n-MDS ordination plot of polysaccharide composition of the bivalve species sampled at the Mondego estuary and at the Ria Formosa lagoon, in winter (2016) and summer (2017) seasons. A, B and C are the groups defined in the n-MDS.

**Table 5**
Results of SIMPER analyses of abundance of sugar residues in polysaccharides showing average similarity and dissimilarity among the species inside each group according to non-metric multidimensional scaling (n-MDS) analysis.

| Group | Average Similarity | Sugar Residues | Av. Abund | | Av. Sim | Sim/SD | Contrib% | Cum.% |
|---|---|---|---|---|---|---|---|---|
| A | 69.94 | Glucose | 4680.44 | | 61.09 | 4.14 | 87.35 | 87.35 |
| | | Xylose | 187.98 | | 3.05 | 2.98 | 4.37 | 91.72 |
| B | 82.97 | Glucose | 1275.13 | | 73.77 | 10.28 | 88.91 | 88.91 |
| | | Ribose | 68.21 | | 2.61 | 1.54 | 3.14 | 92.05 |
| C | 65.55 | Glucose | 441.42 | | 52.29 | 3.28 | 79.76 | 79.76 |
| | | Uronic Acids | 22.40 | | 3.07 | 2.43 | 4.69 | 84.45 |
| | | Ribose | 21.93 | | 3.00 | 1.47 | 4.57 | 89.02 |
| | | Galactose | 18.66 | | 2.24 | 1.41 | 3.42 | 92.44 |
| Groups | Average Dissimilarity | Sugar Residues | Av. Abund | | Av. Diss | Diss/SD | Contrib% | Cum.% |
| A/B | 51.57 | Glucose | 4680.44 | 1275.13 | 43.58 | 2.62 | 84.51 | 84.51 |
| | | Xylose | 187.98 | 53.60 | 2.02 | 2.16 | 3.91 | 88.42 |
| | | Ribose | 125.45 | 68.21 | 1.28 | 1.33 | 2.47 | 90.90 |
| A/C | 78.29 | Glucose | 4680.44 | 441.42 | 67.76 | 5.29 | 86.56 | 86.56 |
| | | Xylose | 187.98 | 27.21 | 2.95 | 2.27 | 3.77 | 90.32 |
| B/C | 51.12 | Glucose | 1275.13 | 441.42 | 40.09 | 2.65 | 78.41 | 78.41 |
| | | Uronic Acids | 73.30 | 22.40 | 3.21 | 0.94 | 6.28 | 84.69 |
| | | Ribose | 68.21 | 21.93 | 2.28 | 1.61 | 4.45 | 89.15 |
| | | Xylose | 53.60 | 27.21 | 2.04 | 1.19 | 4.00 | 93.14 |

ribose between groups B/C.

### 3.4. Fatty acid trophic markers

The analysis of FATMs revealed an omnivorous behaviour in all bivalve species, sampled in both seasons in the Mondego estuary and in the Ria Formosa lagoon, with high input of zooplankton, as seen by DHA, C18:1n-9, C18:2n-6 and C20:1n-9 abundances, high input of phytoplankton, observed in the PUFAs/SFAs, DHA/EPA and C16:1n-7/C16:0 ratios, and low input of bacteria and detritus, assessed with PUFAs/SFAs ratio and the sum of C15:0 and C17:0 (Table 8). In *C. edule*, zooplankton contributed more to the species' omnivorous diet than phytoplankton. Furthermore, in these specimens the bacterial and detritus input was higher in winter, however it contributed less to their diets than the zooplankton and phytoplankton input. The FATMS found in *C. gigas* indicated higher input of phytoplankton, particularly dinoflagellates, than zooplankton, bacteria and detritus. Small and big *M. galloprovincialis* specimens from the Mondego estuary, as well as big size bivalves from the Ria Formosa lagoon, showed higher concentrations of FATMs characteristic of zooplankton and dinoflagellate consumption in both seasons, with low input of bacteria and detritus. *R. decussatus* demonstrated FATMs associated with higher assimilation

**Table 6**
Abundance of monosaccharides, in terms of neutral sugars (μg/g), of the bivalve species sampled in the Mondego estuary (M) and in the Ria Formosa lagoon (RF), in the winter of 2016 (W) and in the summer of 2017 (S).

| Species | | *Cerastoderma edule* | | | | *Crassostrea gigas* | | *Mytilus galloprovincialis* S | | *Mytilus galloprovincialis* B | | | | *Ruditapes decussatus* | | | | *Scrobicularia plana* | | | | *Solen marginatus* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Area | | M | | RF | | RF | | M | | M | | RF | | M | | RF | | M | | RF | | M | | RF | |
| Season | | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S |
| Neutral Sugars | Rhamnose | 1.76 | 1.03 | 39.60 | 1.55 | | | 9.11 | 2.72 | 2.99 | 1.43 | | 0.50 | | 1.45 | | 0.67 | | | | | | | 1.17 | 0.70 |
| | Fucose | 7.23 | 14.59 | 122.16 | 10.87 | | | 27.59 | 21.74 | 28.71 | 3.31 | 8.30 | 5.41 | 11.30 | 14.54 | | 0.36 | 7.65 | 2.67 | | | 0.56 | 0.20 | 0.78 | |
| | Ribose | 1.09 | 21.25 | 48.93 | 36.75 | | 0.98 | 47.60 | 26.52 | 12.78 | 5.15 | 2.14 | 9.80 | 7.09 | 1.27 | 50.11 | 2.12 | 12.27 | 11.20 | 0.88 | 5.97 | 2.37 | 2.12 | | |
| | Arabinose | | 28.51 | 25.41 | 27.41 | | 0.27 | 8.19 | 28.96 | | 26.67 | | | | 33.16 | | 8.57 | 6.32 | 13.77 | 0.36 | 0.76 | 0.21 | 3.56 | | |
| | Xylose | 3.51 | | 96.20 | 9.28 | 1.68 | 0.98 | 146.15 | 83.99 | 1.31 | 3.62 | | | 48.17 | 26.01 | 11.18 | 1.67 | 7.25 | 1.81 | | 0.66 | 2.52 | | | |
| | Mannose | 0.47 | 1.43 | 3.21 | 3.02 | | 0.80 | 6.30 | | | 2.75 | | | | | 2.58 | | 1.65 | 2.63 | | 1.10 | 1.01 | 0.57 | | |
| | Galactose | 0.18 | 0.61 | 16.75 | 4.44 | | | 15.28 | | 3.32 | 0.11 | | | 9.54 | 19.41 | 1.05 | | 2.25 | 2.16 | | 0.61 | | | | |
| | Glucose | 94.34 | 258.52 | 1120.81 | 256.26 | 198.82 | 95.38 | 208.46 | 755.21 | 145.82 | 726.87 | 62.66 | 92.94 | 354.77 | 411.05 | 125.83 | 215.16 | 154.48 | 172.26 | 91.67 | 134.24 | 133.77 | 153.50 | | |
| | **Total FA** | 108.58 | 325.94 | 1473.07 | 349.58 | 200.5 | 98.41 | 468.68 | 919.14 | 194.93 | 769.91 | 73.10 | 108.65 | 430.87 | 506.89 | 190.75 | 228.55 | 191.87 | 206.50 | 92.91 | 143.90 | 141.25 | 161.23 | | |
| | **N** | 7 | 7 | 8 | 8 | 2 | 5 | 8 | 6 | 7 | 8 | 3 | 4 | 5 | 7 | 5 | 6 | 7 | 7 | 3 | 7 | 7 | 6 | | |

of zooplankton, dinoflagellates and diatoms than bacteria and detritus, in the Mondego estuary, while, in the Ria Formosa lagoon, the FATMs present were mainly resultant of dinoflagellate and zooplankton input. Moreover, in the lagoon system, the low PUFAs/SFAs ratio indicated lower input of phytoplankton than detritus by *R. decussatus*, in both seasons. In the Mondego estuary, *S. plana* demonstrated an input of zooplankton higher than the input of phytoplankton in winter, whereas the inverse tendency was observed in summer. This species also showed a considerable input of detritus and bacteria in winter. *S. marginatus*, collected in both study areas, exhibited an omnivorous behaviour in winter, based on the presence of FATMS related to the input of zooplankton, phytoplankton, bacteria, and detritus. Nonetheless, this species revealed an herbivorous behaviour in summer, indicated by the PUFAs/SFAs ratio and the low abundance of C18:1n-9, C18:2n-6 and C20:1n-9. Dinoflagellates were the main food assimilated, which was indicated by DHA abundance and low C16:1n-7/C16:0 ratio.

The two-dimensional n-MDS plot (Fig. 6) showed a distribution according to FATMs abundance and feeding preferences (stress = 0.04). Three groups were defined. Group A comprised the species that presented an omnivorous behaviour with greater phytoplankton consumption, including dinoflagellates and diatoms, than zooplankton consumption. In group B were included the species with omnivorous behaviour, exhibiting higher inclination for phytoplankton consumption, mainly dinoflagellates, than for the consumption of zooplankton. *S. marginatus* specimens collected in summer at both study areas, that showed an herbivorous behaviour, were in group C. ANOSIM analysis indicated a clear segregation of the three groups defined ($R = 0.96$; $p = 0.00$). When comparing pairwise tests, all groups were significantly different ($p \leq 0.05$) and presented high R values, showing good segregation (A/B: $R = 0.95$, $p = 0.00$; A/C: $R = 1$, $p = 0.03$; B/C: $R = 0.96$, $p = 0.01$). SIMPER analysis (Table 9) showed that average similarities were mainly explained by DHA, DHA/EPA, EPA, PUFA/SFA and C18:1n-9 in group A; by DHA/EPA, PUFA/SFA, C16:1n-7/C16:0, DHA and EPA in group B; by PUFA/SFA, C16:1n-7/C16:0 and DHA in group C. The average dissimilarities were mostly explained by DHA, EPA, C18:1n-9, C20:1n-9 and C15:0 + C17:0 between groups A/B; DHA, EPA, DHA/EPA, C18:1n-9 and C20:1n-9 between groups A/C; by DHA/EPA, DHA, PUFA/SFA, EPA and C16:1n-7/C16:0 among groups B/C.

## 4. Discussion

The bivalve species studied revealed diverse biochemical composition, as it was expected for a seafood product (Larsen et al., 2011; Tacon and Metian, 2013). Seasonal and spatial changes in the biochemical composition were highlighted in this study, corroborating the statements from previous studies (Ansell, 1972; Walne and Mann, 1975; Newell and Bayne, 1980; Navarro et al., 1989; Rodríguez-Rúa et al., 2003; Dridi et al., 2007; Martínez-Pita et al., 2012; Matias et al., 2013). This variability could result from several environmental factors, including physical–chemical parameters of the study areas such as temperature and salinity, precipitation, food availability, food composition, pollutants and ecosystem dynamic, as well as from physiological factors, like gender, mobilization of nutrients, energy storage and use during the reproductive cycle. In this study, *M. galloprovincialis* S, *M. galloprovincialis* B and *R. decussatus* from the Mondego estuary and *C. gigas* from the Ria Formosa lagoon demonstrated higher condition index in summer, while *S. plana* and *S. marginatus* from the estuarine system and *C. edule*, *M. galloprovincialis* B, *R. decussatus* and S. *marginatus* from the Ria Formosa lagoon demonstrated higher condition index in winter. *C. edule* from the Mondego estuary showed no differences of condition index between seasons. Despite the condition indices measured in this study, bivalves harvested in summer showed less nutritive value, as the various constituents of the biochemical compositions (proteins, fatty acids and carbohydrates) revealed lower abundances in this season, which could be a possible outcome of the physical–chemical conditions of both systems during this season allied

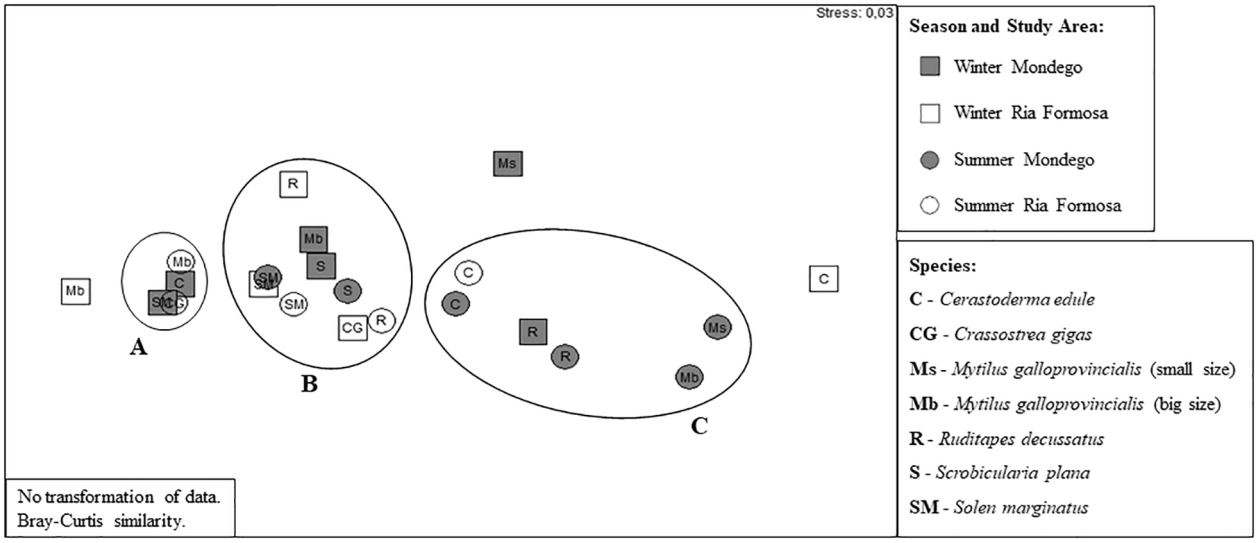


**Fig. 5.** Two-dimensional n-MDS ordination plot of monosaccharide composition of the bivalve species sampled at the Mondego estuary and at the Ria Formosa lagoon, in winter 2016 and summer 2017. A, B and C were the groups defined in the n-MDS.

**Table 7**
Results of SIMPER analyses of monosaccharide abundance, in terms of neutral sugars, showing average similarity and dissimilarity among the species inside each group according to n-MDS analysis.

| Group | Average Similarity | Neutral Sugars | Av. Abund | | Av. Sim | Sim/SD | Contrib% | Cum.% |
|---|---|---|---|---|---|---|---|---|
| A | 92.83 | Glucose | 93.58 | | 90.71 | 27.62 | 97.72 | 97.72 |
| B | 81.16 | Glucose | 159.32 | | 77.16 | 9.73 | 95.07 | 95.07 |
| C | 69.15 | Glucose | 460.45 | | 60.14 | 4.54 | 86.96 | 86.96 |
| | | Arabinose | 24.12 | | 3.42 | 1.22 | 4.95 | 91.91 |
| Groups | Average Dissimilarity | Neutral Sugars | Av. Abund | | Av. Diss | Diss/SD | Contrib% | Cum.% |
| A/B | 31.10 | Glucose | 93.58 | 159.32 | 22.47 | 2.71 | 72.27 | 72.27 |
| | | Ribose | 3.19 | 10.99 | 3.45 | 0.73 | 11.08 | 83.35 |
| | | Fucose | 3.16 | 4.55 | 1.99 | 0.77 | 6.40 | 89.76 |
| | | Arabinose | 0.16 | 3.69 | 1.20 | 0.81 | 3.86 | 93.62 |
| A/C | 65.78 | Glucose | 93.58 | 460.45 | 52.19 | 3.89 | 79.33 | 79.33 |
| | | Arabinose | 0.16 | 24.12 | 4.00 | 1.72 | 6.07 | 85.41 |
| | | Xylose | 1.12 | 28.51 | 3.90 | 1.09 | 5.93 | 91.33 |
| B/C | 48.59 | Glucose | 159.32 | 460.45 | 36.73 | 2.20 | 75.59 | 75.59 |
| | | Xylose | 3.12 | 28.51 | 3.36 | 1.10 | 6.91 | 82.50 |
| | | Arabinose | 3.69 | 24.12 | 3.16 | 1.83 | 6.51 | 89.01 |
| | | Ribose | 10.99 | 16.34 | 2.40 | 1.11 | 4.94 | 93.95 |

with reproductive effort caused by ripe and/or spawning stages. In winter, when, usually, bivalves are enduring the resting stage and/or gametogenesis, the nutritive value was higher as a response to the accumulation of nutrients. The different stages of the reproductive cycle of the bivalve species mentioned previously have already been studied by Navarro et al. (1989) (in *C. edule*), Dridi et al. (2007) (in *C. gigas*), Martínez-Pita et al. (2012) (in *M. galloprovincialis*), Matias et al. (2013) (in *R. decussatus*), Rodríguez-Rúa et al. (2003) (in *S. plana*) and Remacha-Triviño and Anadón (2006) (in *S. marginatus*) and were correlated with the variations of biochemical composition.

Most of the species from the Mondego estuary and the Ria Formosa lagoon showed fatty acid contents more diverse and with higher abundances in winter than in summer. PUFAs and HUFAs, were the main contributors to the FA profile of the studied bivalve species, revealing to be good bioindicators of seasonal variations, followed by MUFAs and SFAs. FAs found in higher quantities in the edible bivalve species studied were described as the main contributors for FA composition in numerous bivalve species in preceding studies (Dridi et al., 2007; Prato et al., 2010; Ezgeta-Balić et al., 2012; Tacon and Metian, 2013; Gonçalves et al., 2016; Mesquita et al., 2018). All bivalve species demonstrated higher protein content in winter, in both study areas. In both systems and seasons, glucose was the most abundant sugar residue found in polysaccharides. Thus, these results indicated that glycogen was the main polysaccharide present in bivalves, which was in accordance with previous researches (de Zwaan and Zandee, 1972; Pérez Camacho et al., 2003; Matias et al., 2013). The maximum concentrations of fatty acids,

**Table 8**
Fatty acid trophic markers (FATMs) (μg/g) of the bivalve species sampled in the Mondego estuary (M) and in the Ria Formosa lagoon (RF), in the winter of 2016 (W) and in the summer of 2017 (S).

| Species | | *Cerastoderma edule* | | | | *Crassostrea gigas* | | *Mytilus galloprovincialis* S | | *Mytilus galloprovincialis* B | | | | *Ruditapes decussatus* | | | | *Scrobicularia plana* | | *Solen marginatus* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Area | | M | | RF | | RF | | M | | M | | RF | | M | | RF | | M | | M | | RF | |
| Season | | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S |
| FATMs | PUFAs/SFAs | 1.57 | 1.71 | 2.29 | 2.32 | 4.15 | 1.91 | 2.22 | 4.04 | 3.24 | 2.27 | 3.42 | 2.07 | 2.14 | 2.33 | 0.28 | 0.86 | 1.06 | 2.11 | 0.87 | 1.44 | 1.91 | 2.04 |
| | DHA | 0.32 | 0.20 | 12.80 | 4.83 | 0.19 | 0.01 | 73.56 | 14.95 | 3.98 | 0.57 | 1.08 | 0.40 | 14.13 | 13.75 | 0.11 | 0.08 | 4.61 | 0.14 | 0.07 | 0.01 | 0.09 | 0.02 |
| | EPA | 0.19 | 0.12 | 6.00 | 2.43 | 0.10 | 0.00 | 21.58 | 8.48 | 1.77 | 0.29 | 0.50 | 0.14 | 2.36 | 4.14 | 0.03 | 0.03 | 0.99 | 0.13 | 0.03 | | 0.05 | |
| | DHA/EPA | 1.74 | 1.61 | 2.13 | 1.98 | 1.97 | 3.80 | 3.41 | 1.76 | 2.24 | 1.94 | 2.14 | 2.79 | 5.98 | 3.32 | 4.24 | 2.43 | 4.64 | 1.10 | 2.65 | | 2.07 | |
| | C16:1n-7/C16:0 | 1.03 | 1.13 | 0.13 | 0.45 | 0.42 | 0.82 | 0.07 | 0.82 | 0.07 | 0.20 | 0.25 | 0.62 | 0.25 | 0.25 | 0.39 | 0.67 | 0.28 | 0.58 | 2.04 | 0.72 | 0.39 | 0.46 |
| | C15:0 + C17:0 | 0.10 | 0.05 | 0.77 | 0.33 | 0.01 | 0.01 | 3.40 | 0.62 | 0.21 | 0.09 | 0.08 | 0.12 | 0.67 | 0.70 | 0.33 | 0.08 | 0.70 | 0.03 | 0.07 | 0.01 | 0.04 | 0.01 |
| | C20:1n-9 | 0.02 | 0.01 | 0.63 | 0.32 | 0.02 | | 24.02 | 5.63 | 0.48 | 0.08 | 0.19 | 0.04 | 0.57 | 0.67 | | | 0.52 | 0.06 | 0.01 | | 0.01 | 0.00 |
| | C18:1n-9 | 0.03 | 0.02 | 1.01 | 0.46 | 0.03 | 0.01 | 6.44 | 0.98 | 0.26 | 0.04 | 0.09 | 0.03 | 1.56 | 0.95 | 0.01 | 0.01 | 1.54 | 0.04 | 0.02 | 0.00 | 0.01 | 0.00 |
| | C18:2n-6 | 0.01 | 0.02 | 0.41 | 0.08 | 0.02 | 0.00 | 3.27 | 1.80 | 0.15 | 0.06 | 0.03 | 0.02 | 0.19 | 0.17 | | 0.02 | 0.20 | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 |

proteins and polysaccharides observed in winter and the decrease to lower values during summer could be a response to the environmental and physiological conditions of the bivalves (Dridi et al., 2007). High monosaccharide abundances in summer were not expected, since in this period, the nutritive stress is supposed to be higher, due to energetic investments in the reproductive success of the bivalve species (Matias et al., 2013). However, these results could be a consequence of the environmental conditions of the study areas. Glucose was the main monosaccharide found, as expected. The other monosaccharides found, in much lower abundances than glucose, are synthesized by marine algae, seaweeds and some microorganisms and can be metabolized by bivalves and enter their biochemical composition (Ahmed et al., 2014; Kang et al., 2015).

Since bivalves play a pivotal role in the transfer of nutrients and energy throughout the marine trophic webs, the FATMs present in the species used in this study revealed valuable insight on their feeding habits. The FATMs accessed were characteristic of omnivorous behaviour, indicating a dietary preference not only for phytoplankton and zooplankton, but also for detritus and bacteria. The results regarding the feeding behaviour were in line with previous researches that concluded marine bivalves with different feeding strategies, such as the filter-feeder *M. galloprovincialis* (Prato et al., 2010), the suspension-feeder *C. edule* and the deposit filter-feeder *S. plana* (Gonçalves et al., 2016; Mesquita et al., 2018), revealed to have omnivorous preferences. However, for *C. gigas*, our findings differed from another study, conducted in a different estuary, that revealed a tendency for herbivory (Kasim and Mukai, 2009). The variation of the contribution of different dietary components over the year in each species, and, consequently, the dietary changes, like the ones observed in *S. marginatus* in winter and summer, could be explained by seasonal changes of food availability in the ecosystem throughout the year, dietary preferences in case of similar abundance of preys and/or different filtration rates (Ezgeta-Balić et al., 2012).

In conclusion, *M. galloprovincialis* and *R. decussatus* were the species that demonstrated higher biochemical composition in the Mondego estuary, while in the Ria Formosa lagoon were *C. edule*, *C. gigas* and *R. decussatus*, presenting higher fatty acid, especially essential fatty acids, total protein, polysaccharide and monosaccharide contents. Thereby, these species are pointed out as being the best choices for a healthy human diet and being confirmed as a reliable choice to local farmers and stakeholders for harvesting and production in aquacultures. This study highlights the importance of bivalves as marine resources to humans and the fact that seasonal and spatial changes may influence their biochemical composition. Since bivalves have central roles in the ecosystems and any perturbation may affect their biological processes, consequently disturbing other organisms at distinct trophic levels, it is imperative to have a sustainable exploration of these resources, as well as identify species with potential to be used in aquaculture, in order to maintain their natural stocks and avoid the breakdown of economic valuable species.

## CRediT authorship contribution statement

**Daniela C.C. Silva:** Data curation, Writing - original draft. **João M. Neto:** Conceptualization, Writing - review & editing. **Cláudia Nunes:** Writing - review & editing. **Fernando J.M. Gonçalves:** Writing - review & editing. **Manuel A. Coimbra:** Writing - review & editing. **João C. Marques:** Writing - review & editing. **Ana M.M. Gonçalves:** Conceptualization, Supervision, Data curation, Writing - review & editing.

## 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.

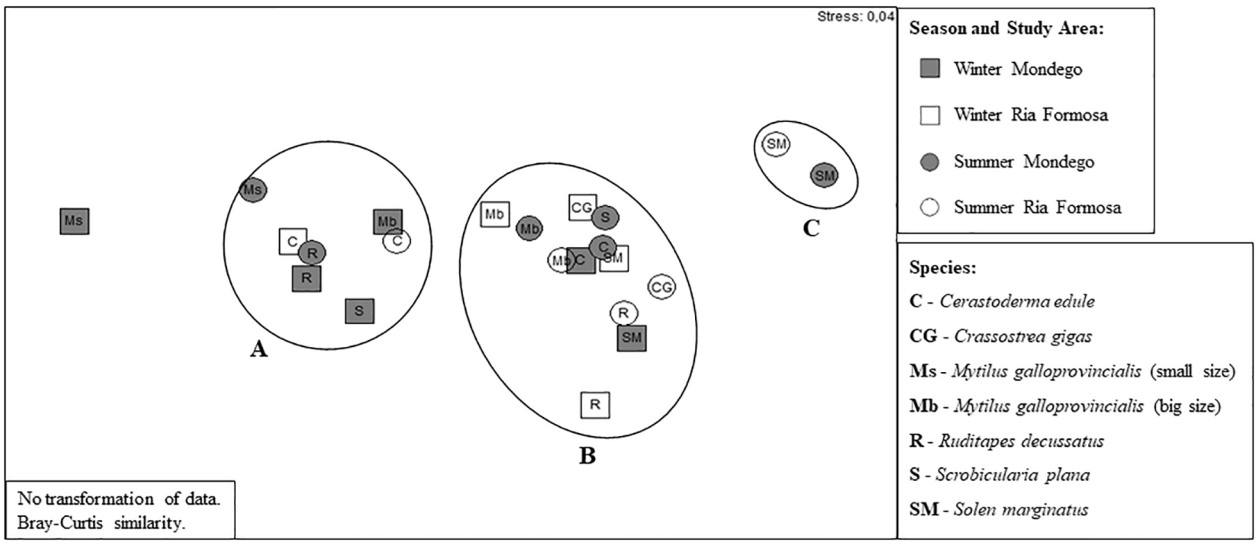


**Fig. 6.** Two-dimensional non-metric multidimensional scaling (n-MDS) ordination plot of fatty acid trophic markers composition of the bivalve species sampled at the Mondego estuary and at the Ria Formosa lagoon, in winter and summer seasons. A, B and C were the groups defined in the n-MDS.

**Table 9**
Results of SIMPER analyses of fatty acid trophic markers abundance showing average similarity and dissimilarity among the species inside each group according to n-MDS analysis.

| Group | Average Similarity | Fatty Acid Trophic Markers | Av. Abund | | Av. Sim | Sim/SD | Contrib% | Cum.% |
|---|---|---|---|---|---|---|---|---|
| A | 82.15 | DHA | 9.86 | | 21.18 | 5.25 | 25.79 | 25.79 |
| | | DHA/EPA | 3.15 | | 12.73 | 4.94 | 15.49 | 41.28 |
| | | EPA | 3.74 | | 11.97 | 4.79 | 14.57 | 55.85 |
| | | PUFAs/SFAs | 2.49 | | 11.72 | 5.21 | 14.27 | 70.12 |
| | | C18:1n-9 | 0.96 | | 6.58 | 4.00 | 8.01 | 78.13 |
| B | 80.25 | DHA/EPA | 2.37 | | 26.72 | 5.90 | 33.29 | 33.29 |
| | | PUFAs/SFAs | 1.93 | | 21.46 | 3.60 | 26.74 | 60.04 |
| | | C16:1n-7/C16:0 | 0.71 | | 12.84 | 3.55 | 16.00 | 76.03 |
| | | DHA | 0.27 | | 6.21 | 2.78 | 7.74 | 83.77 |
| | | EPA | 0.13 | | 4.15 | 2.15 | 5.17 | 88.94 |
| C | 88.48 | PUFAs/SFAs | 1.74 | | 49.92 | – | 56.43 | 56.43 |
| | | C16:1n-7/C16:0 | 0.59 | | 28.37 | – | 32.07 | 88.50 |
| | | DHA | 0.02 | | 4.27 | – | 4.82 | 93.32 |
| Groups | Average Dissimilarity | Fatty Acid Trophic Markers | Av. Abund | | Av. Diss | Diss/SD | Contrib% | Cum.% |
| A/B | 45.03 | DHA | 9.86 | 0.27 | 14.89 | 4.01 | 33.06 | 33.06 |
| | | EPA | 3.74 | 0.13 | 8.69 | 2.98 | 19.29 | 52.35 |
| | | C18:1n-9 | 0.96 | 0.03 | 4.64 | 3.02 | 10.31 | 62.66 |
| | | C20:1n-9 | 1.26 | 0.04 | 4.58 | 1.80 | 10.18 | 72.84 |
| | | C15:0 + C17:0 | 0.57 | 0.08 | 2.82 | 2.68 | 6.27 | 79.11 |
| A/C | 69.92 | DHA | 9.86 | 0.02 | 20.05 | 6.10 | 28.68 | 28.68 |
| | | EPA | 3.74 | 0.00 | 12.58 | 4.48 | 17.99 | 46.68 |
| | | DHA/EPA | 3.15 | 0.00 | 12.44 | 3.71 | 17.80 | 64.48 |
| | | C18:1n-9 | 0.96 | 0.00 | 6.35 | 3.57 | 9.08 | 73.56 |
| | | C20:1n-9 | 1.26 | 0.00 | 3.24 | 2.34 | 8.93 | 82.48 |
| B/C | 42.44 | DHA/EPA | 2.37 | 0.00 | 20.44 | 4.33 | 48.15 | 48.15 |
| | | DHA | 0.27 | 0.02 | 4.33 | 1.62 | 10.20 | 58.35 |
| | | PUFAs/SFAs | 1.93 | 1.74 | 4.17 | 1.15 | 9.83 | 68.18 |
| | | EPA | 0.13 | 0.00 | 4.14 | 2.12 | 9.76 | 77.94 |
| | | C16:1n-7/C16:0 | 0.71 | 0.59 | 2.85 | 1.20 | 6.71 | 84.64 |

## Acknowledgements

This work was financed by national funds through FCT – Foundation for Science and Technology, I.P., within the scope of the projects UIDB/04292/2020 – MARE – Marine and Environmental Sciences Centre and UIDP/50017/2020 + UIDB/50017/2020 (by FCT/MTCES) granted to CESAM – Centre for Environmental and Marine Studies. This work was also developed within the scope of the projects CICECO-Aveiro Institute of Materials (UIDB/50011/2020 & UIDP/50011/2020), QOPNA (UID/QUI/00062/2019) and LAQV-REQUIMTE (UIDB/50006/2020), financed by national funds through the FCT/MEC and, when appropriate, co-financed by FEDER under the PT2020 Partnership Agreement. This work was also funded by national funds (OE), through FCT, I.P., within the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19. This research was partially supported by PORBIOTA, E-Infrastructure Portuguese Information and Research in Biodiversity (POCI-01-0145-FEDER-022127), supported by Competitiveness and Internationalization Operational Programme and Regional Operational Programme of Lisbon, through FEDER, and by the

Portuguese Foundation for Science and Technology (FCT), through national funds (OE). Ana M. M. Gonçalves acknowledges University of Coimbra for the contract IT057-18-7253.

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