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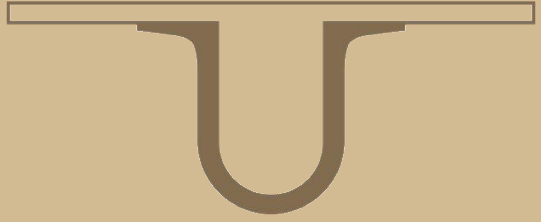

Daniela Carolina da Costa Silva

ECONOMIC POTENTIAL OF MARINE BIVALVES OF THE PORTUGUESE COAST: ECOLOGICAL AND BIOCHEMICAL CHARACTERIZATION

Dissertação de Mestrado na área científica de Ecologia, orientada pela Doutora Ana Marta Mendes Gonçalves e pelo Professor Doutor João Carlos de Sousa Marques e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

Setembro de 2018

Departamento de Ciências da Vida

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Resumo

Os bivalves estão distribuídos por diversos habitats, incluindo estuários que são ecossistemas extremamente produtivos, e desempenham funções importantes nas redes tróficas e nos processos biológicos que ocorrem nos ecossistemas. Assim, como outros recursos marinhos, fazem parte da dieta dos seres humanos desde que estes começaram a pescar. Estes recursos possuem elevado valor nutricional, devido ao alto teor em proteína e baixo teor em gordura, estando o seu consumo associado a vários benefícios para a saúde, nomeadamente o bom desenvolvimento do sistema nervoso e a redução da incidência de doenças cardiovasculares. Os recursos marinhos, incluindo bivalves, que são muito apreciados pelos seres humanos, representam um importante valor económico, estando sob pressão devido à crescente procura. Assim, é importante uma exploração sustentável e equilibrada, baseada no conhecimento da composição bioquímica das espécies aquáticas de modo a compreender o seu potencial e valor nutricional.

O presente estudo foi realizado em Portugal, onde existe um dos maiores consumos de recursos marinhos do mundo. Seis espécies de bivalves marinhos com valor comercial foram recolhidas em duas áreas distintas, no estuário do Mondego e na lagoa da Ria Formosa, e em duas estações do ano, no Inverno de 2016 e no Verão de 2017. Os objetivos do estudo foram: 1) determinar a composição bioquímica de cada espécie em relação à composição total de proteína, ao perfil em ácidos gordos e em hidratos de carbono; 2) identificar potenciais variações espaciais e sazonais entre espécies recolhidas em cada uma das áreas de estudo e das estações do ano; e 3) determinar as preferências alimentares de cada espécie em ambas as áreas de estudo e estações do ano.

Os resultados indicaram composições bioquímicas diversificadas em todas as espécies, em que a composição total de proteína é a principal componente, seguida pelo conteúdo em ácidos gordos, em particular os ácidos gordos essenciais DHA e EPA, pelo glicogénio e pela glucose, que são os principais polissacarídeos e açúcares neutros, respetivamente, encontrados em todas as amostras. Em geral, todas as espécies demonstraram uma tendência para a omnivoria, com apenas a espécie *S. marginatus* a demonstrar um claro comportamento herbívoro no Verão. Apesar de *M. galloprovincialis* e *R. decussatus* apresentarem elevado valor nutricional no estuário do Mondego, em ambas as estações, este foi mais pronunciado no Inverno. Já na Ria Formosa, *C. edule* e *R. decussatus* apresentaram valor nutritivo mais elevado em ambas

as estações do ano, enquanto *C. gigas* apresentou maior valor nutricional apenas no Verão.

Palavras-chave: bivalves, valor económico, composição bioquímica, valor nutricional, estuário do Mondego, lagoa da Ria Formosa

Abstract

Bivalves are widely distributed through diverse habitats, including estuaries 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 like the good development of the nervous system and the reduction of incidence of cardiovascular diseases. 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 food preferences 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 neutral sugar, 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.

Keywords: bivalves, economic value, biochemical composition, nutritional value, Mondego estuary, Ria Formosa lagoon.

1. Introduction

1.1. Ecological, trophic and economic importance of bivalves in estuarine and coastal ecosystems

Marine ecosystems, and especially estuaries and coastal lagoons, are among the most important environments in the world due to several ecological and economical features. These include high productivity, high biodiversity of species, existence of key-species, ecosystem services and high commercially value of marine resources used in food, pharmaceutical and cosmetic industries (Cardoso et al., 2008; Barbier et al., 2011; Ahmed *et al*., 2014). Bivalves, considered highly valuable marine resources to humans, are usually found in transitional waters systems, like estuaries, and in coastal waters systems, like coastal lagoons. According to the European Water Framework Directive, transitional waters systems can be classified as “surface water bodies in the vicinity of river mouths partly saline due to their proximity to coastal waters but significantly influenced by freshwater flows”, while coastal waters systems are classified as ”surface water on the inward side of a line at a distance of one nautical mile on the seaward side from which the territorial waters are measured and extending, where appropriate, up to the outer limit of transitional waters” (Bettencourt *et al*., 2003; Conde *et al*., 2013). These ecosystems have primary production through the entire year, but it increases in the warmer seasons (spring and summer) since the higher temperatures increase the biological metabolism, consequently increasing primary production (McLusky *&* Elliott, 2004). Estuaries and coastal lagoons have been increasingly affected by perturbations like industrialization, urban development, tourism, agriculture and climate changes. It is known that global warming is characterized by a progressive temperature increment (∼0.89◦C from 1901 to 2012) and by the occurrence of extreme climate events like heat waves, droughts or floods. These events can provoke changes in temperature, salinity and hydrodynamic conditions on aquatic ecosystems. Consequently, this affects the physiological processes, behavior and mortality of bivalves and other aquatic species (Philippart *et al*., 2011; Fuji, 2012; Verdelhos *et al*., 2015a). Thus, a wide number of compounds, including metals, polyaromatic hydrocarbons, fertilizers, pesticides, pharmaceutical and personal care products, are found in the discharged water released from industrial and municipal effluents to the estuaries and, even at low concentrations, contribute to a negative impact in the aquatic communities, including bivalves (Cravo *et al*., 2012; Fuji, 2012). Bivalves are unable to distinguish between food particles and

floating detritus of identical size. Therefore, at some point, occurs the uptake of heavy elements, microbial pathogens or marine bio-toxins (McLusky *&* Elliott, 2004; Almeida, 2015). In ecotoxicological studies, bivalve molluscs have been widely used as biomonitors, since they have the ability to concentrate contaminants from the environment in their tissues – bioaccumulation – and are able to respond to pollutant exposure and to climate changes, thus they reflect the habitat conditions (Rainbow *&* Phillips, 1993; Nasci *et al*., 2000; Cravo *et al*., 2012). In addition, many bivalves have a relative long lifespan, which allows for frequent sampling during a considerable period in order to have a representative response to the bioaccumulation. Despite a considerable accumulation of pollutants in their tissues without harmful impacts to the bivalves, when the pollutants' concentration decreases in the environment, some species can eliminate the contaminant from their own tissues (Belabed *&* Soltani, 2018). The contaminants present in the estuarine and coastal waters are particularly dangerous when they enter at the food chain by consumption of contaminated bivalves (Coelho *et al*., 2006; Cravo *et al*., 2012). Consumption of bivalves, as well as other seafood, should be moderate and cautious, to prevent the excessive intake of toxic compounds (e.g. mercury, microalgae, and bacteria). It should not exceed three portions of seafood per week (Potasman *et al*., 2002; Ström *et al*., 2011; Almeida, 2015).

Bivalve species play important roles in the trophic web because they can connect primary producers to consumers and are the major prey of gastropods, starfish, crabs, fish, birds, and mammals (Dame, 2011; Almeida, 2015; Gonçalves *et al*., 2016). Bivalve molluscs play central roles in ecosystem processes. They represent the biggest portion of biomass in estuaries and they play a key role controlling the ecosystem' structure and functions. Bivalve populations also have a strong influence on the benthic processes of other organisms. Evidence from previous studies show that bivalves' burrowing activity increases the oxygen penetration in the sediments, which stimulates the microbial metabolism (Levinton, 1995; Dame, 2011). Bivalve populations, like mussel beds, can modify light, temperature, water circulation, sediment loading and deposition patterns. Furthermore, the shells provide refuge and habitat for a wide group of organisms (Dame, 2011).

Bivalves, a class of organisms included in the phylum Mollusca, are characterized by presenting a shell composed by two valves. They have a wide geographical distribution, from estuaries, shallow coastal waters, rocky shores and reefs to rivers and lakes (Vaugh *et al*., 2001; Gosling, 2003; Dame, 2012). Most species bury themselves in sediment of estuaries for protection, but they can also lie on the sea floor or attach

themselves to rocks or other hard surfaces in costal shores. Many factors, including spawning, food availability, type of substratum, depth, light, temperature, salinity, wind, rainfall, river input, nutrients and population density affects the bivalves' growth rates (Almeida, 2015). Bivalves can be filter-feeders or suspension-feeders. They absorb substantial amounts of suspended material from the water, including phytoplankton, detritus from a mixture of sources, bacteria and zooplankton, being possible to determine the food sources when trophic markers are examined (Prato *et al*., 2010).

The life cycle of marine bivalves starts with the external fertilization of gametes and is divided in two stages. The pre-settlement is the first stage and it comprises the larval period. This stage is of vital importance for the dispersal of the species. But while being scattered through the water column, mixed with other microorganisms, the planktonic larvae are exposed to a wide variety of predators, therefor they have high mortality at this stage of life. The post-settlement begins with a metamorphosis from larva to juvenile and it continues during the adult period. At this point, bivalves stop being a part of the plankton and become members of the benthonic community. It is during the post-settlement stage that reproduction occurs. Pre-settlement and post-settlement stages organisms are spatially and temporally separated throughout the most of their respective stage. This reduces the probability of larvae mortality due to adult feeding behavior (Gosling, 2003)

Bivalves are a type of seafood very appreciated by humans and have been included in our dietary patterns for a long time, especially bivalve species occurring in shallow waters. Historically, it is demonstrated that our ancestors, who lived nearby coastal regions and other water bodies, started to fish and gather these resources for consumption as well as for the treatment or prevention of numerous diseases and health problems. It was known since the ancient Greek civilization that some bivalves had beneficial effects to human health and their consumption was encouraged by the great physicians of that time (Voultsiadou *et al*., 2009; Colonese *et al*., 2011). Bivalves as well as other molluscs, fish, and crustaceans are the main seafood products consumed and traded around the world and are associated with a high nutritious value. These products have been traded more than any other food commodity in the world (Tacon *&* Metien, 2013). The seafood dietary choices made in each country are related to their economy, culture, geographical location, globalization and religion (Kearney, 2010). Naturally, the local species are the most consumed in each country, but the demand for these resources is so high that some of the most appreciated species are transported through long distances from their native production sites to their consumers or are introduced in

other regions to replace stocks of indigenous species severely depleted by over-fishing or disease. With an increase of the consumption of bivalves and other seafood products, the over-exploitation of the species stocks also increases. This impact allied with climate change, invasive species and coastal development are major threats to the conservation of marine species. The consumption of seafood resources needs to be balanced with the sustainability and good management of marine stocks and exploration rates need to decrease to prevent the collapse of highly economic valuable species (Dulvy *et al*., 2003; Kearney, 2010; Tacon *&* Metian, 2013; Almeida *et al*., 2015).

The major seafood markets in the world are in the Asiatic, American and European continents (Table 1). Recent data published by FAO (Food and Agriculture Organization of the United Nations) shows that in 2013, Asia was responsible for producing 67.18% of the global production of fishery commodities and 85.65% of global production of bivalves. China is the biggest producer in Asia, producing 32.57% of the global production of seafood and 75.32% of the global production of bivalves in 2013. For a while, China has been the major producer of seafood products, mainly through aquaculture. It is the largest seafood exporter in the world, since 2002, and the third largest importer, since 2012. The exports of fishery products reached €16.91 billion and the imports reached €7.26 billion in 2015. It also has one of the highest consumptions of these commodities in the world (34.67kg *per capita* in 2013) (FAO, 2018a). Japan is the second biggest bivalve producer in the word, producing 4.84% of the global bivalve produced in 2013. Republic of Korea was the 4th largest producer of bivalves in this year (FAO, 2016; FAO, 2017).

In America was produced 15.67% of the global seafood production and 8.67% of the global bivalve production in 2013. USA (United States of America) was the biggest producer of seafood commodities and bivalves in America, and the 3rd largest producer of bivalves in the world (4.29% of global production of bivalves). The exportation trades of fishery commodities in USA reached €5.06 billion, while importation trades reached €16.98 billion in 2015. USA is one of the largest importers in the world. The consumption per capita in USA in 2013 was 21.5kg/year (FAO, 2018a). Chile was the second biggest producer of bivalves in America and the 5th major producer worldwide (1.85% of global bivalve production). Canada was the 8th biggest country in the world to produce bivalves in 2013 (FAO, 2016; FAO, 2017).

The European continent produced, in 2013, 11.78% of global seafood commodities and 4.90% of bivalves produced worldwide. In terms of bivalve production in this year, France was the main producer, representing 26% of the European bivalve

production and 1.28% of the global bivalve production, making it the 6th largest producer in the world. The consumption per capita of seafood commodities was 33.48kg in 2013 (FAO, 2018a). France exports reached €1.39 billion, while imports reached €4.91 billion in 2015. In 2013, Spain was the second largest producer of bivalves in Europe, which represented 23.50% of the European bivalve production. It was the 7th largest producer in the world with 1.15% of the global bivalve production. The consumption per capita of seafood commodities was 42.38kg in the same year (FAO, 2016; FAO, 2017, FAO, 2018a).

Table 1. World's largest producers of bivalves and their respective bivalve and fishery production, consumption per capita of fishery commodities, and international trades in 2013 and 2015. Data available in FAO (2017). Bivalve production***** includes the groups of oysters, mussels, scallops, clams, and cockles. Fishery production****** includes the groups of marine fishes, aquatic mammals, crustaceans, molluscs and aquatic plants.

	Bivalve production* (1 000 tonnes)		**Fishery production** (1 000 tonnes)**		**Consumption *per capita* (kg/year)**		**Exportations (EUR million)**		**Importations (EUR million)**	
Year	**2013**	**2015**	**2013**	**2015**	**2013**	**2015**	**2013**	**2015**	**2013**	**2015**
Asia	13 079	11 639	89 244	35 252	23.10	-	-	-	-	-
China	11 502	10 586	43 263	46 074	34.67	-	16 739	16 909	6 838	7 254
Japan	739	215	4 702	4 586	48.60	-	1 698	1 629	13 123	11 531
Rep. of Korea	324	373	3 111	3 301	52.78	-	1 523	1 298	3 122	3 726
America	1 324	953	20 822	19 630	14.30	-	-	-	-	-
USA	654	480	5 310	5 209	21.51	-	5 109	5 064	16 256	19 980
Chile	282	249	3 290	3 189	12.49	-	4 271	4 122	353	367
Canada	135	71	998	1 013	22.52	-	4 739	4 030	2 422	2 302
Europe	748	732	15 648	16 414	21.90	-	-	-	-	-
France	195	133	698	634	33.48	-	1 559	1 389	5 574	4 909
Spain	175	240	1 175	1 243	42.38	-	3 381	3 214	5 474	5 517
Portugal	7	13	206	199	53.76	-	921	954	1 651	1 665
Oceania	112	99	1 392	1 564	24.80	-	-	-	-	-
Africa	6	5	5 735	6 197	9.90	-	-	-	-	-
World	15 270	13 428	132 843	139 056	19.70	-	119 391	114 151	114 301	109 293

In 2013, Portugal was the 9th country with the largest production of bivalves in Europe, representing 0.96% of the European production and 0.05% of the global bivalve production. In the same year, it was produced 1.32% of the European seafood commodities and 0.16% of the global seafood production. Portugal exports reached €0.95 billion in 2015, while imports reached €1.66 billion. As a consequence of the geographical location and culture drivers of the country, Portugal has the 3rd highest seafood consumption *per capita* in Europe (53.76kg in 2013), after Iceland and Faroe Islands (FAO, 2018a). It surpasses the major producers, France and Spain, which consumed 33.48kg and 42.38kg *per capita*, respectively, in the same year (FAO, 2016; FAO, 2017). With a coastline of 1 214 km (including Azores and Madeira islands) and an Exclusive Economic Zone (EEZ) of 1 727 408 km^2, which makes this country the 4th with the largest EEZ within EU and the 20th with the largest EEZ worldwide, there are several commercial important bivalve species that are very appreciated by the Portuguese consumers. The consumption of bivalves along with the consumption of diverse fish, cephalopod and crustacean species, contribute to the high seafood supply on this country (Pham *et al*., 2013; Leitão *et al*., 2014; Shon *et al*., 2015).

The countries mentioned above, in spite of being the largest producers of bivalves in the world and some being the major seafood producers, are largely dependent of importations of seafood from other locations to fulfil the demand for these commodities (Swartz *et al*., 2010). Oysters, mussels, scallops and clams are the most traded bivalves worldwide and represent a high commercial value (Table 2). Pacific cupped oyster (*Crassostrea gigas*) is the major species cultivated throughout the world. It is native from Japan and was introduced in many regions of the world, including France, which was the main exporter in 2017. American cupped oyster (*Crassostrea virginica*) is an important species cultured in North America, especially in United States of America. Currently, China is the major producer of mussels in the world with a production of nearly 800 000 tonnes every year. Blue mussel (*Mytilus edulis*) is amongst all the mussel species produced in this country. China is followed by Chile, with a massive aquaculture industry of Chilean blue mussel (*Mytilus chilensis*), which is mostly exported to other countries, like Spain and United States of America, that appreciate this bivalve species. The Ribbed mussel (*Aulacomya atra*) is a native species and is very appreciated by Chilean consumers. Spain is the third major producer of mussels, where the Mediterranean mussel (*Mytilus galloprovincialis*) and the Blue mussel (*M. edulis*) are the main species produced. Another mussel that is intensively traded worldwide, from New Zealand to Japan, Australia, Spain, Germany and France, is the Green-lipped mussel (*Perna*

canaliculus) native from New Zealand. The United States of America and China are the main scallop producers and consumers, where the Yesso scallop (*Patinopecten yessoensis*) is the species of choice to farm. Another important scallop species is the Peruvian scallop (*Argopecten purpuratus*), that is cultured in Peru and it is mainly exported to the United States of America. Clams are largely produced and consumed in China and, in a smaller scale, all over the world. The principal clam produced in China is the Japanese carpet shell (*Ruditapes phillipparum*). Amongst the main importers are Japan and Republic of Korea. Besides the Asiatic countries, European countries also appreciate these species and introduced it in their waters. It is the major contributor to clam landings in Europe, after the decrease of the native clam Grooved carpet shell (*Ruditapes decussatus*), which is still produced in France, Spain, Portugal and in the African country, Algeria (FAO, 2017; FAO, 2018b).

Table 2. World production of major bivalve groups and their estimated economic value in 2013 and 2015. Data available in FAO (2017).

	Capture fisheries				**Aquaculture**			
Year	**2013**		**2015**		**2013**		**2015**	
Units	**1 000 t**	**EUR million**	**1 000 t**	**EUR million**	**1 000 t**	**EUR million**	**1 000 t**	**EUR million**
Oysters	135	110	147	122	4 953	3 506	5 322	3 507
Mussels	97	35	119	44	1 736	2 817	1 878	2 646
Scallops	747	1 216	573	942	1 868	2 887	2 082	2 777
Clams, cockles	579	581	611	502	5 157	4 436	5 392	4 547
World total	1 558	1 842	1 450	1 610	13 714	13 646	14 674	13 478

Among the most valuable bivalve species in Portugal are the Common cockle (*Cerastoderma edule*), the Pacific cupped oyster (*Crassostrea gigas*), the Mediterranean mussel (*Mytilus galloprovincialis*), the Grooved carpet shell (*Ruditapes decussatus*), the Peppery furrow shell (*Scrobicularia plana*) and the Grooved razor shell (*Solen marginatus*), which were the species selected to work in this thesis. The Common cockle, *Cerastoderma edule* (Figure 1A), is a bivalve species from the family Cardiidae and is distributed from North Africa to Northern Norway. It is found on the east coast of the Atlantic Ocean. It occurs naturally in tidal flats, estuaries and bays (Freitas *et al*., 2014; Gonçalves *et al*., 2016). *C. edule* organisms are suspension-feeders and are buried at

shallow depth in the sediment, making the harvest an easy task. This species is frequently used in the Portuguese gastronomy. The most important characteristics of the common cockle morphology are the thick shells with an oval shape; equilateral valves; two cardinal teeth in each valve; right valve with two anterior lateral teeth and other two posterior teeth; left valve with an anterior and a posterior lateral tooth; similar anterior and posterior adductor muscle scars; pallial line without pallial sinus; approximately 25 radial lines crossed by concentric ridges; the radial lines vanish when they reach the pallial line (Hayward *et al*., 1996).

The Pacific cupped oyster, *Crassostrea gigas* (Figure 1B), is a bivalve species from the family Ostreidae and is native from Asia. Originally, it was introduced in France, in the 1960s and spread through all the European coastal territory, after the drastic decrease of the Portuguese oyster (*C. angulata*) due to a disease (Grizel *&* Héral, 1991; Fabioux *et al*. 2002, Forrest *et al*., 2009). Like other introduced species, it can outcompete the native bivalve species. For this reason, the native *C. angulata* only exists in Mira and Sado estuaries in Portugal (Fabioux *et al*. 2002). *C. gigas* has a huge economic value and the production of the Pacific oyster represents the most important aquaculture industries in the world (FAO, 2016). This oyster is typically found in estuaries but can also occur in intertidal and subtidal zones. Usually, they are attached to hard or rocky surfaces. But when their ideal habitat is scarce, they can attach to muddy or sandy substrate. This species is characterized by large, rounded, radial folds that are extremely irregular and sharp; The right valve is concave and bigger than the left valve. The colour of the shells varies from pale white to off-white (Hayward *et al.*, 1996).

The Mediterranean mussel, *Mytilus galloprovincialis* (Figure 1C), is distributed along the south-west Great Britain, Atlantic French, Spanish, Portuguese and Morocco coasts and is also present in the Mediterranean Sea (Martinez-Pita *et al*., 2012). This is a sessile species from the family Mytilidae, that appears naturally from middle tide level to shallow sublittoral areas, forming dense mantles that cover the rocky substrate, but it is also found in sandy bottoms (Hayward *et al.*, 1996). Among the features that are typical of the mussel morphology are the equivalve and inequilateral shells; anterior adductor muscle reduced or absent; colour of the valves can range from bluish to purplish, while the mantle edge is purplish; the ventral margin is often concave (Hayward *et al*, 1996).

The Grooved carpet shell*, Ruditapes decussatus* (Figure 1D), is widespread in coastal waters of Europe and Mediterranean Sea. It has a high economic value which makes this species one of the most harvested in estuaries and coastal lagoons. It occurs on muddy sands and gravel on the lower coasts and shallow sub-littoral zones. *R.*

decussatus is extensively produced and harvested in the Ria Formosa lagoon where clam farming represents an important economic sector (Hayward *et al.*, 1996; Cravo *et al*., 2012). This bivalve, that belongs to the Veneridae family, have large oval shells with a checkered effect (radial and longitudinal lines); a cardinal tooth in the center of the left valve and two cardinal teeth in the right valve, one at the centre and the other at the posterior side; yellowish or orange shells and blue near the hinge; short but broad pallial sinus (Hayward *et al.*, 1996).

The Peppery furrow shell, *Scrobicularia plana* (Figure 1E), is from the family Semelidae and is a dominant deposit filter-feeder, naturally seen buried in mud or in brackish waters in intertidal and subtidal areas of estuaries, lagoons, and bays. The distribution goes from Norway to the Mediterranean and West African regions (Essink *et al*., 1991; Hayward *et al*., 1996; Mouneyrac *et al*., 2008; Verdelhos *et al*., 2015b). This species has flattened and thin shells with oval-shape and numerous concentric lines and grooves; equivalve and inequilateral valves (slightly to the posterior side); the colour of the shells goes from opaque to greyish white; the hinge has two cardinal teeth on the right valve and one on the left; the inner ligament is within a chondrophore; pallial sinus is almost circular; cruciform muscles are present but the respective scars are indistinct. The star pattern left in the sand by the siphon is characteristic of this species (Hayward *et al.*, 1996).

The Grooved razor shell, *Solen marginatus* (Figure 1F), belongs to the Solenidae family, lives buried in sand or muddy sand in low intertidal and subtidal areas of estuaries and coastal lagoons, from Norway to the Mediterranean and Black Sea, and West Africa. When it feels threatened, it has the capacity for a rapid vertical burrowing into the sand (Hayward *&* Ryland, 1998; da Costa *et al*., 2011). Among the adaptative features of this bivalve mollusc are the characteristic elongated and semi-cylindrical valves; equivalve and inequilateral valves; one or two cardinal teeth in each valve and lateral teeth present or absent; the characteristic groove in the anterior margin of each valve; anterior adductor muscle scar is bigger than posterior; pallial sinus present (Hayward *et al.*, 1996).

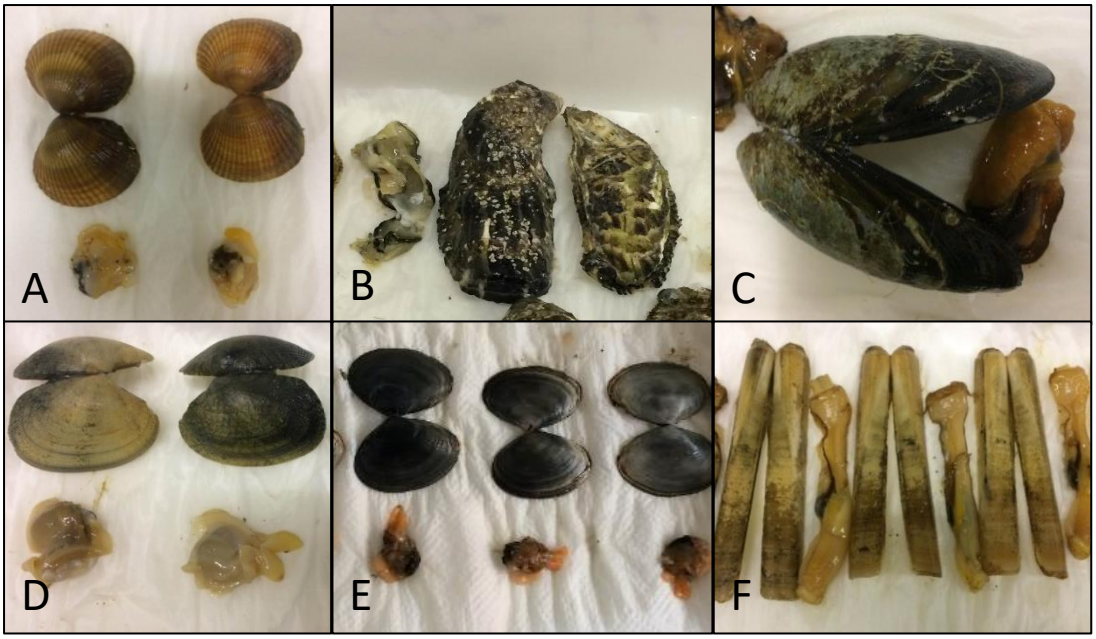

Figure 1. Shells and edible portions of the commercially valuable bivalves selected for this study: *Cerastoderma edule* (A), *Crassostrea gigas* (B)*, Mytilus galloprovincialis* (C), *Ruditapes decussatus* (D), *Scrobicularia plana* (E) and *Solen marginatus* (F). (Photo courtesy of the supervisor Ana Marta Gonçalves)

1.2. Biochemical Composition of Bivalves

In terms of biochemical composition, bivalves are considered a healthy and nutritious food source, as well as other seafood commodities, because of their low fat and high protein contents. It is demonstrated that seafood products are constituted by several essential fatty acids and amino acids, vitamins (A, B, D and E), minerals (Ca, Mg and Zn), and trace elements (iodine, fluorine and trivalent chromium) (Karakoltsidis *et al.*, 1995; Larsen *et al*., 2011; Tacon *&* Metian, 2013; Almeida *et al*., 2015). This is justified by the fact that bivalves have a diverse fatty acid (FA) profile, including saturated fatty acids (SFA), without double bonds, and unsaturated fatty acids (UFA), with one or more double bonds. UFAs can be divided in two groups, considering the number of double bonds. Monounsaturated fatty acids (MUFA) have one double bond and polyunsaturated fatty acids (PUFA) have two or more double bonds. Highly unsaturated fatty acids (HUFA) are an important subset of the last group and are those fatty acids with a chain of 20 or more carbon atoms and 3 or more double bonds. Arachidonic acid (C20:4n-6 or ARA), eicosapentanoic acid (C20:5n-3 or EPA) and docosahexanoic acid (C22:6n-3 or DHA) are HUFA 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 present in bivalves' biochemical composition are the α-linolenic acid (C18:3n-3 or ALA) and linoleic acid (C18:2n-6 or LA). Bivalves and most of the other seafood are known to have high concentrations of these essential fatty acids in their tissues (Ezgeta-Balić *et al*., 2012; Gonçalves *et al*., 2016; Gonçalves *et al*., 2017a, b).

Several studies demonstrate the beneficial properties of UFAs to human health, and specially the advantages of consuming highly unsaturated fatty acids from seafood. ARA is important to a good development and performance of the nervous system, the skeletal muscle and immune systems and, in addition, it has a selective tumoricidal action and potential antimicrobial properties against a variety of infections (Tallima *&* El Ridi, 2017). EPA and DHA are important for neuronal, retinal, and immune development in infants. These essential fatty acids help to reduce the incidence of cardiovascular diseases, cancer, atherosclerosis, dysfunctional behaviours and neurological diseases (Ruxton *et al*., 2004; Riediger *et al*., 2009; Larsen *et al*., 2011; Swanson *et al*., 2012; Tacon *&* Metian, 2013; Ibarguren *et al*., 2014). The consumption of monounsaturated, polyunsaturated and essential fatty acids is highly recommended. On the opposite side, the consumption of saturated fatty acids is associated with negative cardiovascular effects, thus it must be avoided (Ibarguren *et al*., 2014; Briggs *et al*., 2017). Besides their important role in nutrition as an energy source, fatty acids are the main constituent of cell membranes and enter in several biochemical pathways (Ibarguren *et al*., 2014; Liu *et al*., 2015; Gonçalves *et al*., 2016;).

Usually, bivalves have a protein content higher than the total lipid (fat) content. In fact, most seafood commodities present higher protein quantities than meat from terrestrial animals, and its quality may exceed that of meat as well. Proteins are important biomolecules composed of one or more long chains of amino acids. Among the amino acids in the nutritional composition of bivalves, glutamate and aspartate are the non-essential amino acids that occur with higher concentrations. Meanwhile, leucine, lysine and arginine are the main essential amino acids present in their tissues and, similarly to what occur with essential fatty acids, are obtained through feeding (Brown *et al*., 1996; Tacon *&* Metian, 2013). In a recent research, the analysis and description of the complete amino acid profile of an economic valuable bivalve species was achieved (Aru *et al*., 2017).

In terms of carbohydrate composition, bivalves have glycogen as the main polysaccharide in their body, which is expected since it is the main source of energy stored used by bivalves and other animals. Glycogen is composed by several units of

glucose residues. Therefore, glucose is the primary neutral sugar present in the bivalves' tissues. Other sugar residues are present in these organisms' tissues, like uronic acids. These compounds can be found in uronic acid-containing polysaccharides, like hyaluronic acid. Several polysaccharides have been isolated and characterized from different families of molluscs, including bivalves. Nevertheless, previous studies do not specify the complete carbohydrate profile composition of bivalves but state that they are important for gamete formation and maintaining adult condition during periods of nutritive stress (de Zwaan *&* Zandee, 1972; Camacho *et al*., 2003; Volpi & Maccari, 2003; Liu et al., 2016)

Although seasonal changes in the biochemical composition of bivalve molluscs, in their natural habitat, have been reported by several authors, none of the studies were conducted in Portuguese aquatic systems, taking into account the commercially valuable bivalve species and their complete biochemical composition (Ansell, 1972; Ansell, 1974; Walne & Mann, 1975; Newell & Bayne, 1980; Robert *et al*., 1993; Aru *et al*., 2017). Changes in the organisms' biochemical composition are proved to be associated with the state of sexual maturity and with the energy supply, provided either by food ingestion or by previously stored reserves. The biochemical composition is also influenced by the environmental surroundings (Bayne, 1976; Zandee *et al*., 1980; Navarro *et al*., 1989; Camacho *et al*., 2003).

1.3. Objectives

Considering the central role of bivalve molluscs in the estuarine ecosystems and in the marine food webs, as well as their economic value and the weather conditions at the Portuguese coast for the success reproduction and development of these organisms, it is greatly important to determine and assess the biochemical composition of most consumed species from different regional coastal areas in Portugal. So, it could be possible to identify which species are the best nutritional food source, if their nutritional value correspond to the respective economic value and compare both parameters (the nutritional and economic values) of these species from the west and south coasts of Portugal in order to assess the best quality product and price relationship. Thus, this work aimed to 1) determine the biochemical profiles of several appreciated bivalve species: the Common cockle (*Cerastoderma edule*), the Mediterranean mussel (*Mytilus galloprovincialis*), the Grooved carpet shell (*Ruditapes decussatus*), the Peppery furrow

shell (*Scrobicularia plana*), the Pacific oyster (*Crassostrea gigas*) and the Grooved razor shell (*Solen marginatus*), from two distinct geographic areas, the Mondego estuary and the Ria Formosa lagoon, collected in winter (December 2016) and in summer (June 2017), 2) identify seasonal and spatial variations and 3) assess food preferences of the studied bivalves species by determining fatty acid trophic markers.

The null hypotheses tested were a) the fatty acid profiles are equal between species and do not present spatial and seasonal variations; b) the total protein content is equal between species and does not show spatial and seasonal variations; c) the polysaccharide residues profiles are equal between species and do not reveal spatial and seasonal variations; d) the neutral sugar profiles are equal between species and do not present spatial and seasonal variations; e) the bivalve species show the same food preferences.

2. Materials and Methods

2.1. Studied Areas

Samples were collected in two different coastal areas of Portugal: Mondego estuary and Ria Formosa lagoon (Figure 2). The Mondego estuary, located near Figueira da Foz city (40º08' N, 8º50' W), is a mesotidal system covering an area of 8.6 km^2 along the West Atlantic coast. According to European Water Framework Directive, this estuary is considered a transitional water system. It comprises two channels, north and south, separated by the Murraceira island. The north channel is deeper (4-8m in high tides; tidal range 1-3m) and more hydrodynamic than the south channel. The south channel is shallower (2-4m in high tides; tidal range 1-3m). 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).

The Ria Formosa lagoon is located in the south coast of Portugal (36º58' N, 8º02' W to 37º03' N, 7º32' W). This is a shallow mesotidal system composed by multiple channels, salt marshes and tidal flats, covering an area of approximately 84km^2. Sandy barrier-islands protect this system from the Atlantic Ocean. This is considered a shallow coastal system, since the mean depth is 3 m, and the tides have a strong impact in the lagoon. However, it is also influenced by the input of freshwater from several intermittent rivers and streams (Ribeiro *et al*., 2008; Cravo *et al*., 2012; Guimarães *et al*., 2012).

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

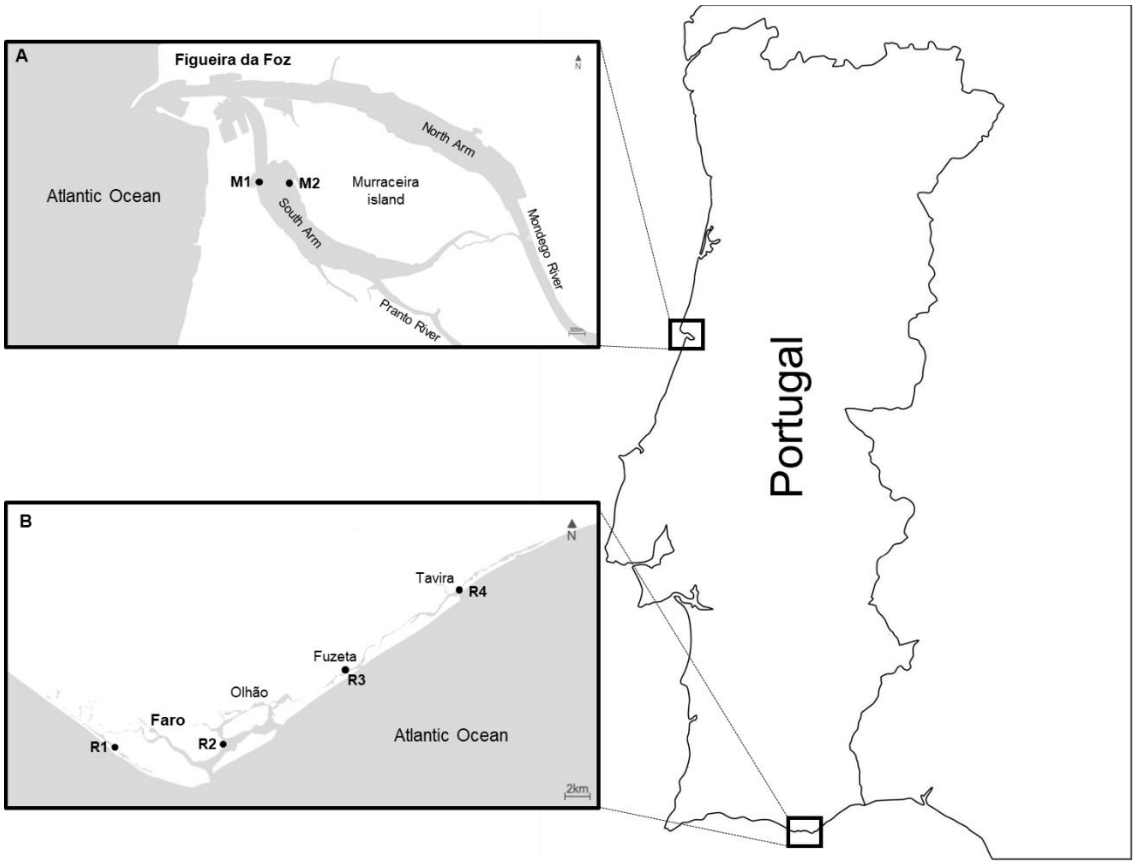

Figure 2. Bivalve species sampled in two distinct studied areas from the Portuguese coast: the Mondego estuary (A) and the Ria Formosa lagoon (B). Black dots represent the sampling stations: M1 (*M. galloprovincialis*) and M2 (*C. edule*, *R. decussatus, S. plana* and *S. marginatus*) in the Mondego estuary, R1 (*S. marginatus*), R2 (*M. galloprovincialis*), R3 (*C. edule* and *R. decussatus*) and R4 (*C. gigas*) in the Ria Formosa lagoon.

2.2. 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. Since bivalves are usually buried in the substrate, they were caught by digging holes in the intertidal mud.

In the Mondego estuary, the organisms were harvested in the south channel, at low tide. *C. edule*, *R. decussatus*, *S. plana* and *S. marginatus* were collected in the sampling station M2 (Figure 2) in the sandy intertidal substrate on the Murraceira island margin (40°07'49.1 N, 8°50'40.7W). *M. galloprovincialis* was sampled in the sampling station M1 (Figure 2) in the small harbour "*Núcleo Piscatório da Cova-Gala*" (40°07'57.6N, 8°51'17.1W) on the opposite margin. For M. *galloprovincialis,* adults with

different sizes (small size and big size) were sampled in winter and summer. To collect *S. marginatus*, the ‘‘salting method’’ was used. It consists in spreading salt on the substrate, where are the burrowing entrances of these bivalves, and once the specimens detect its presence they leave the burrow gallery and are easily collected (da Costa *&* Martínez-Patiño, 2009). Samples were divided according to the species, put inside a plastic bag, and brought to the MAREFOZ lab, in a cold box (4ºC), for processing (Table 3). It was not possible to sample 15 replicates for two species, *R. decussatus* and *S. marginatus*, in the winter campaign and for the species *R. decussatus*, in the summer campaign, due to their low abundance in the estuary. During the sample processing, the replicates were divided equally into sub-replicates, in order to have enough samples to proceed to biochemical analysis.

In the Ria Formosa lagoon *C. edule* and *R. decussatus* (sampling station R3 in Figure 2B), *C. gigas* (sampling station R4 in Figure 2B), *M. galloprovincialis* (sampling station R2 in Figure 2B) and *S. marginatus* (sampling station R1 in Figure 2B) were harvested by artisanal fisherman’s and brought to the lab in the University of Algarve, where the samples were processed (Table 4). Since *Crassostrea gigas* is a species with higher economic value in the south of Portugal, it was sampled instead of *Scrobicularia plana*, which does not occur in great abundance in this region. The samples were transported inside a cold box (4ºC) to the MAREFOZ laboratory, where the samples were stored.

During the sample processing, every organism was weighed, with and without valves. Length and width of the valves were measured. The edible portion of each bivalve sample was stored inside a Falcon tube (50 mL) and preserved at -80ºC until the biochemical analysis.

Table 3. Biometric parameters measured (mean ± standard error) during the sample processing and respective sample size (n = number of organisms) for species from the Mondego estuary. S* means small organisms; B** means big organisms.

	Species	Sample size (N)	Total weight (g)	Organism weight (g)	Height (mm)	Length (mm)
Winter	*C. edule*	15	12.52 ± 0.69	2.71 ± 0.14	36.69 ± 0.34	41.57 ± 0.32
	M. galloprovincialis (S*)	15	0.80 ± 0.05	0.23 ± 0.02	16.29 ± 0.26	31.57 ± 0.33
	M. galloprovincialis (B**)	15	3.43 ± 0.12	1.21 ± 0.05	26.51 ± 0.42	52.67 ± 0.80
	R. decussatus	3	28.18 ± 7.53	6.60 ± 2.00	52.07 ± 3.07	36.80 ± 1.85
	S. plana	15	3.34 ± 0.17	1.14 ± 0.06	25.93 ± 0.42	42.93 ± 0.31
	S. marginatus	5	15.95 ± 2.19	10.64 ± 1.36	90.16 ±2.17	18.16 ± 1.07
Summer	*C. edule*	15	12.83 ± 0.86	2.83 ± 0.26	32.99 ± 0.69	36.74 ± 0.81
	M. galloprovincialis (S*)	15	2.14 ± 0.14	0.69 ± 0.06	20.50 ± 0.46	37.37 ± 0.82
	M. galloprovincialis (B**)	15	8.60 ± 0.24	3.50 ± 0.10	32.99 ± 0.45	62.41 ± 0.74
	R. decussatus	3	21.67 ± 2.42	5.50 ± 0.29	35.93 ± 1.37	47.43 ± 4.10
	S. plana	15	5.40 ± 0.35	1.70 ± 0.14	33.27 ± 0.65	42.56 ± 0.90
	S. marginatus	15	12.37 ± 0.33	8.03 ± 0.25	15.35 ± 0.17	96.84 ± 1.04

Table 4. Biometric parameters measured (mean ± standard error) during the sample processing and respective sample size (n = number of organisms) for species from the Ria Formosa lagoon. B** means big organisms.

	Species	Sample size (N)	Total weight (g)	Organism weight (g)	Height (mm)	Length (mm)
Winter	*C. edule*	15	4.43 ± 0.19	0.95 ± 0.04	23.86 ± 0.31	28.06 ± 0.37
	C. gigas	15	58.58 ± 2.93	6.75 ± 0.54	46.90 ± 1.72	88.82 ± 2.28
	M. galloprovincialis (B**)	15	10.83 ±0.64	2.35 ± 0.16	28.39 ± 1.87	59.27 ± 1.06
	R. decussatus	15	8.40 ± 0.45	2.14 ± 0.15	27.69 ± 0.48	39.44 ± 0.65
	S. marginatus	15	8.65 ± 0.54	5.25 ± 0.32	14.57 ± 0.28	86.66 ± 2.12
Summer	*C. edule*	15	6.27 ± 0.27	0.95 ± 0.06	27.42 ± 0.30	31.83 ± 0.43
	C. gigas	15	62.60 ± 2.86	10.55 ± 0.79	51.27 ± 1.09	88.96 ± 1.54
	M. galloprovincialis (B**)	15	15.37 ± 0.94	3.17 ± 0.24	33.65 ± 0.75	66.18 ± 1.31
	R. decussatus	15	13.34 ± 0.80	3.22 ± 0.20	32.75 ± 0.63	43.72 ± 0.83
	S. marginatus	15	13.02 ± 0.61	7.36 ± 0.35	16.50 ± 0.19	97.66 ± 1.24

2.3. Biochemical Analysis

2.3.1. Fatty Acid Analysis

The edible portion of the bivalve (soft tissue) was entirely used in the fatty acid extraction analysis. The methodology implemented for total lipids extraction and methylation to fatty acid methyl esters (FAMEs) followed the protocol described in Gonçalves *et al*. (2012). The trans-esterification to 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 6890N Network Gas Chromatograph (Agilent Technologies, Santa Clara, CA, USA), equipped with a DB-FFAP capillary column (30m long × 0.25mm i.d. × 0.1 µm film thickness; Agilent Technologies, Santa Clara, CA, USA), associated to a 5973N Mass Selective Detector (Agilent Technologies, Santa Clara, CA, USA) at 70 eV electron impact mode, scanning the range m/z 40-500 in 1s cycle in full scan mode acquisition. The carrier gas He had a 4.4mL 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 250ºC, lined with a splitless glass liner of 4.0mm i.d. Each run had a 42.53-min duration. The injection temperature was 220ºC and the oven temperature was programmed to start at 80ºC, increase to 160ºC at a 25ºC min^{-1} rate, increase to 190ºC at a 2ºC min^{-1} rate, increase to 230ºC min^{-1} at a 40ºC min^{-1} rate, and finally holding for 5 min. The detector starts operating 4 min after injection, corresponding to solvent delay. The injector ion source and transfer line were maintained at 220ºC and 280ºC, respectively. FAMEs were identified by comparison with the retention times and mass spectra of authentic standards and database available (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).

2.3.2. 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 minutes at 4ºC 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. Protein quantification was carried out using a Thermo Scientific Multiskan ® EX Microplate reader (Thermo Scientific, Waltham, MA, USA).

2.3.3. Carbohydrate Analysis

Carbohydrate analysis of bivalve tissue comprised the quantification of polysaccharides, neutral sugars (monosaccharides), and total uronic acid content. The remaining pellets obtained after the centrifugation for fatty acid extraction, mentioned above, were stored in vials at -80ºC until further analysis. For polysaccharide analysis, samples were subjected to hydrolysis followed by reduction and acetylation, as described in Coimbra *et al*. (1996). Neutral sugar analysis samples were not subjected to hydrolysis but followed the same protocol for reduction and acetylation. The alditol acetate derivates obtained in the polysaccharide and neutral sugar 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 (30m length × 0.25mm i.d. × 0.15µm 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 220ºC and the oven temperature was set to increase from 200ºC to 220ºC at a 40ºC min^{-1} rate, stabilize at 220ºC for 7 min, and increase to 230ºC at 20ºC min^{-1} rate, finally maintaining this temperature for 1 min. The carrier gas was H_2, at a flow rate of 1.7mL min^{-1}. Quantification of sugars was obtained by comparison of the sugar chromatographic peaks to the peaks 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. The process was stopped, after 1 hour since the

beginning, to transfer 0.5mL from the culture tubes, that contained the samples for polysaccharide analysis, to new culture tubes. A solution of sodium borate 50mM prepared in sulfuric acid 96% was added to each tube. m-Phenylphenol (MPP) was the dye reagent added to each tube. Samples were distributed throughout microplates and absorbance was read at 520nm, using a BioTek™ Eon Microplate Spectrophotometer (Winooski, VT, USA). Total uronic acid content was obtained through calibration curves created by comparison with different concentrations of the galacturonic acid standard.

2.4. Fatty Acid Trophic Markers

Fatty acid trophic markers 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. Bivalves are considered to be mainly herbivores and phytoplankton (diatoms and dinoflagellates) their primary food source, but traces of zooplankton, bacteria and detritus can be found in their tissues (Ezgeta-Balić *et al*., 2012). Polyunsaturated fatty acids are associated with a diet rich in phytoplankton whereas saturated fatty acids are associated with a consumption of detritus (Volkmann *et al*., 1989; Fahl *&* Kattner, 1993). High quantities of DHA in bivalves are associated with a consumption of dinoflagellates while EPA is related to a consumption of diatoms. Therefore, in bivalves the DHA/EPA ratio expresses the dietary preference between diatoms and dinoflagellates (Budge & Parrish, 1998). Another ratio that can be used to address which type of phytoplankton bivalves are feeding on is C16:1n-7t/C16:0. The monounsaturated fatty acid C16:1n-7t is abundant in diatoms while the saturated fatty acid appears in diatoms (Graeve *et al*., 1994 a b, John *&* Lund, 1996). 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).

2.5. Statistical Analysis

Samples were randomly collected for all biochemical analysis and formed independent groups of variables. A multivariate statistical analysis with the PRIMER-6 software was performed to examine the fatty acid, polysaccharide residues and neutral sugar profiles for discriminatory information about spatial and seasonal variations (Clarke *&* Gorley, 2006). Non-metric multidimensional scaling (nMDS) 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 – fatty acid, polysaccharide residues and neutral sugar – influences both similarities and dissimilarities within and between sample groups. The similarities and dissimilarities were verified through a similarity percentage analysis routine (SIMPER) (Clarke *&* Warwick, 1994). Total uronic acid content values obtained were included in the polysaccharide statistical analysis, since they were considered residues of polysaccharides. The same multivariate analysis was applied with the same purpose to fatty acid trophic markers. Total protein of the bivalves studied 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

Fatty acid (FA) profiles were described in terms of biochemical abundance for each bivalve species sampled in the Mondego estuary (Table 5) and in the Ria Formosa lagoon (Table 6), in winter and summer seasons. In both studied areas and seasons, several fatty acids remained abundant among the bivalves' tissues from all species. In general, the most abundant SFAs were C16:0, C17:0 and C18:0. C16:1 n-7 cis, C17:1 n-8 cis, C20:1 n-9 cis and C22:1n-9 cis were the most abundant MUFAs. All species were rich in PUFAs, particularly in HUFAs such as ARA (C20:4 n-6 cis), EPA (C20:5 n-3 cis) and DHA (C22:6 n-3 cis), which are all essential fatty acids. α-Linolenic acid (C18:3 n-3 cis or ALA) and linoleic acid (C18:2 n-6 cis or LA) were other essential fatty acids that entered the FA composition of the studied bivalves, but in lower concentrations.

Table 5. Abundance of fatty acids (µg/g) of the bivalve species *C. edule*, *M. galloprovincialis* S (small size), *M. galloprovincialis* B (big size), *R. decussatus*, *S. plana* and *S. marginatus* sampled in the Mondego estuary in two different seasons (winter 2016 and summer 2017).

Species	*C. edule*		*M. galloprovincialis* S		*M. galloprovincialis* B		*R. decussatus*		*S. plana*		*S. marginatus*	
Season / **Fatty Acids** (µg/g)	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
C10:0												
C11:0												
C12:0							0.012				0.007	
C13:0	0.007	0.001	1.644	0.042	0.006	0.003	0.024				0.007	
C14:0	0.023	0.014	3.867	0.511	0.086	0.025	0.237	0.341	0.177	0.016	0.002	0.001
C15:0	0.033	0.009	1.219	0.262	0.061	0.025	0.203	0.149	0.135	0.008	0.021	0.000
C16:0	0.135	0.066	29.193	4.531	1.379	0.256	4.465	4.848	1.592	0.071	0.034	0.008
C17:0	0.069	0.041	2.185	0.361	0.144	0.060	0.464	0.549	0.566	0.021	0.046	0.007
C18:0	0.064	0.043	7.202	1.244	0.283	0.051	2.017	1.234	0.883	0.034	0.006	0.003
C20:0	0.018	0.048						0.863	2.627			
C21:0	0.020		3.016		0.021	0.048	0.127		0.683		0.012	
C22:0	0.040	0.022	1.452	0.637	0.212	0.018	0.540	0.706	0.145			
C23:0	0.014	0.006	1.966	0.557	0.094		0.506	0.346	0.177	0.006	0.008	
C24:0							0.464					
Total SFA	0.423	0.250	51.744	8.145	2.286	0.486	9.059	9.036	6.985	0.156	0.143	0.019
C14:1n-5t	0.011	0.004	0.282	1.066	0.048	0.012	0.059	0.142	0.096	0.005	0.006	0.000
C15:1n-5c	0.038	0.014	1.238	0.104	0.060	0.141	0.206	0.28	0.135	0.005	0.014	0.004
C16:1n-7t	0.139	0.074	2.028	3.691	0.080	0.050	1.132	1.205	0.451	0.041	0.068	0.006
C17:1n-8c	0.184	0.074	3.871	0.646	0.764	0.075	1.086	1.006	0.481	0.035	0.147	0.023
C18:1n-9t	0.015	0.007	3.178	0.984	0.134	0.043	1.074	0.771	1.062	0.024	0.010	0.001
C18:1n-9c	0.019	0.016	3.265		0.124		0.482	0.178	0.478	0.014	0.010	0.001
C20:1n-9c	0.020	0.013	24.018	5.627	0.483	0.078	0.565	0.667	0.520	0.057	0.005	
C22:1n-9c	0.027	0.016	9.353	2.598	0.309	0.051	1.535	1.532	0.053	0.004		
C24:1n-9c												
Total MUFA	0.453	0.218	47.233	14.716	2.002	0.450	6.139	5.781	3.276	0.185	0.260	0.035
C18:2n-6t		0.004		1.800								
C18:2n-6c (LA)	0.011	0.014	3.266		0.144	0.055	0.191	0.168	0.203	0.009	0.005	0.000
C18:3n-6t									0.224			0.003
C18:3n-3c (ALA)	0.012	0.015	1.529	1.382	0.107	0.037	0.299	0.391	0.211	0.019	0.006	0.003
C20:2n-6c	0.017	0.017	1.730	1.665	0.653	0.116		0.784				
C22:2n-6c	0.055	0.023	3.389	1.510	0.206	0.032	0.434	0.430	0.133	0.002	0.012	0.005
Total PUFA	0.095	0.073	9.914	6.357	1.110	0.240	0.924	1.773	0.771	0.030	0.023	0.011
C20:3n-7c	0.023			0.732	0.065		0.866	0.134				0.006
C20:4n-6c (ARA)	0.043	0.036	10.001	2.365	0.472		1.130	1.213	1.006	0.026		
C20:5n-3c (EPA)	0.185	0.123	21.582	8.477	1.772	0.293	2.362	4.137	0.993	0.130	0.027	
C22:6n-6c (DHA)	0.322	0.197	73.563	14.954	3.976	0.569	14.133	13.75	4.609	0.142	0.072	0.010
Total HUFA	0.573	0.356	105.146	26.528	6.285	0.862	18.491	19.234	6.608	0.298	0.099	0.016
Total FA	1.544	0.897	214.037	55.746	11.683	2.038	34.613	35.824	17.640	0.669	0.525	0.081
N	26	25	24	23	25	21	26	24	24	20	21	17

Table 6. Abundance of fatty acids (µg/g) of the bivalve species *C. edule*, *C. gigas*, *M. galloprovincialis* B (big size), *R. decussatus* and *S. marginatus* harvested in the Ria Formosa lagoon in two different seasons (winter 2016 and summer 2017).

Species	*C. edule*		*C. gigas*		*M. galloprovincialis* B		*R. decussatus*		*S. marginatus*	
Season / **Fatty Acids** (µg/g)	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
C10:0										
C11:0			0.004							
C12:0			0.003							
C13:0	0.026		0.003			0.007	0.003	0.001		
C14:0	0.920	0.474	0.004	0.001	0.037	0.009	0.027	0.004	0.011	0.001
C15:0	0.265	0.057	0.003	0.001	0.045	0.011	0.112	0.006	0.025	0.001
C16:0	4.331	1.461	0.057	0.007	0.341	0.120	0.104	0.089	0.026	0.010
C17:0	0.508	0.273	0.008	0.006	0.034	0.111	0.220	0.070	0.011	0.007
C18:0	2.203	0.574	0.014	0.004	0.133	0.049	0.019	0.031	0.012	0.006
C20:0	0.778	0.382			0.032	0.028				
C21:0			0.009		0.010		0.016		0.005	
C22:0	1.092	0.647	0.003		0.042	0.010	0.007	0.009		
C23:0	0.671	0.263			0.036	0.015		0.003		
C24:0						0.009				
Total SFA	10.794	4.131	0.108	0.019	0.710	0.369	0.508	0.213	0.090	0.025
C14:1n-5t	0.213	0.121	0.004	0.001	0.026	0.003	0.006	0.002	0.011	0.001
C15:1n-5c	1.053	0.374	0.028	0.003	0.117	0.039	0.048	0.025	0.027	0.003
C16:1n-7t	0.577	0.657	0.024	0.006	0.086	0.074	0.041	0.060	0.010	0.005
C17:1n-8c	0.662	0.509	0.222	0.043	0.358	0.455	0.007	0.224	0.066	0.021
C18:1n-9t	0.511	0.309	0.008	0.002	0.037	0.019	0.011	0.009	0.004	0.001
C18:1n-9c	0.495	0.146	0.023	0.003	0.052	0.009		0.003	0.006	0.000
C20:1n-9c	0.633	0.322	0.019		0.187	0.039			0.007	0.002
C22:1n-9c	0.868	0.493	0.009		0.182	0.085	0.024	0.036	0.010	
C24:1n-9c										
Total MUFA	5.012	2.931	0.337	0.058	1.045	0.723	0.137	0.359	0.141	0.033
C18:2n-6t			0.016	0.002				0.008		0.000
C18:2n-6c (LA)	0.408	0.079			0.033	0.024		0.013	0.008	0.005
C18:3n-6t		0.082								0.001
C18:3n-3c (ALA)	0.682	0.169	0.018		0.020	0.013		0.003	0.010	0.006
C20:2n-6c	0.502	0.190	0.016		0.304	0.018		0.007		
C22:2n-6c	2.094	0.576	0.091		0.055	0.028	0.008	0.003		
Total PUFA	3.686	1.096	0.141	0.002	0.412	0.083	0.008	0.034	0.018	0.012
C20:3n-7c					0.009					0.012
C20:4n-6c (ARA)	2.250	1.249	0.020	0.015	0.426	0.141		0.033	0.018	0.003
C20:5n-3c (EPA)	5.999	2.432	0.096	0.004	0.504	0.142	0.025	0.034	0.046	
C22:6n-6c (DHA)	12.800	4.827	0.189	0.014	1.080	0.395	0.107	0.082	0.094	0.021
Total HUFA	21.049	8.508	0.305	0.033	2.019	0.678	0.132	0.149	0.158	0.036
Total FA	40.541	16.666	0.891	0.112	4.186	1.853	0.785	0.755	0.407	0.106
N	24	24	25	15	25	25	17	23	19	19

The two-dimensional n-MDS plot (Figure 3) showed a separation of samples based on fatty acid concentration and composition (stress = 0.02), not being possible to separate these by species, site or season. Four groups were defined. Group A contained the bivalve species that had the less diversified and the lowest abundance in FA, including *C. gigas* and *S. marginatus* from Ria Formosa and *S. marginatus* from the Mondego estuary, all species collected in summer. Group B comprised the species that had a significant higher abundance on FA than group A, including *C. edule* and S. marginatus collected in winter at the Mondego estuary; *C. edule*, *M. galloprovincialis* B and *S. plana* sampled in summer at the Mondego estuary; *C. gigas*, *M. galloprovincialis* B and *S. marginatus* collected in winter at the Ria Formosa lagoon and *M. galloprovincialis* B and *R. decussatus* sampled in summer at the lagoon system. Group C was formed by the species that had a significant higher abundance in FA than the previous groups, including *M. galloprovincialis* B and *S. plana* collected in winter at the Mondego estuary and *C. edule* sampled in summer at Ria Formosa. Group D included the species that presented the highest abundance in FA from all groups formed, including *R. decussatus* collected in winter and summer at the Mondego estuary, *M. galloprovincialis* S and *C. edule* sampled in summer at the Mondego estuary and in winter at Ria Formosa, respectively. *M. galloprovincialis* S, collected in winter at the Mondego estuary, was not included in group D because of the higher FA abundance in comparison with the species included in it, whereas *R. decussatus,* collected in winter at the Ria Formosa lagoon, was not included in group B because of the distinct FA content in comparison with the species included at that group.

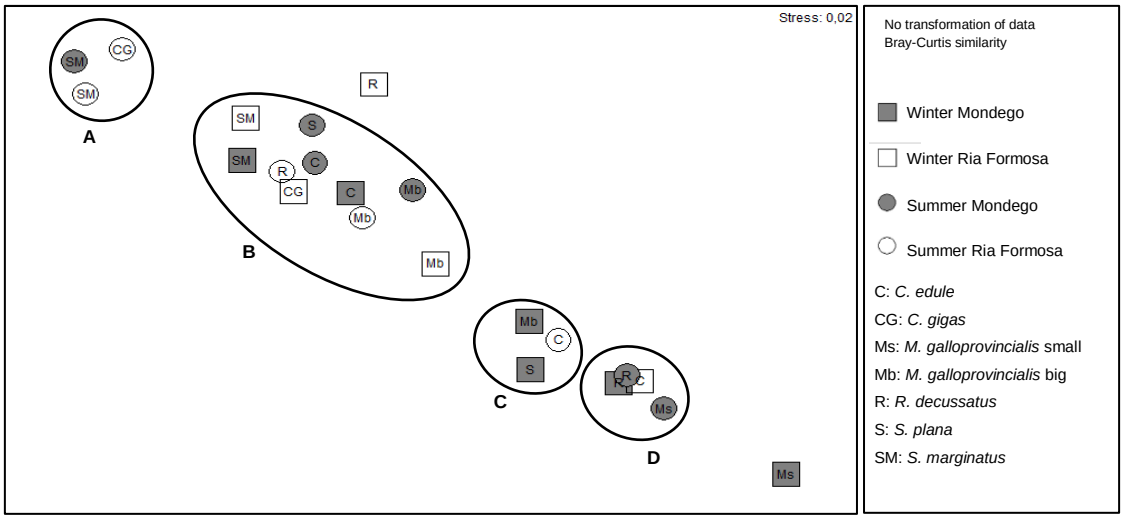

Figure 3. Two-dimensional non-metric multidimensional scaling (n-MDS) ordination plot of fatty acid composition of the bivalve species sampled at the Mondego estuary and at the Ria Formosa lagoon, in winter and summer seasons. A, B, C and D were the groups defined in the n-MDS.

The ANOSIM analysis indicated a clear separation of the groups defined (R = 0.909; p = 0.001). The null hypothesis a) the fatty acid profiles are equal between species and do not present spatial and seasonal variations was rejected. 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 = 0.936, p= 0.003; A/D: R = 1, p = 0.029; B/C: R = 0.931, p = 0.003; B/D: R = 0.997, p = 0.001; C/D: R = 1, p = 0.029). The groups A and C (R = 1, p = 0.1) had strong segregation, but were not significantly different. SIMPER analysis showed that at group A the fatty acids that explained 66.72% of the group similarity, in decreasing order of importance, were: C17:1n-8c; DHA; C16:0; C17:0; C16:1n-7t; C18:0; C15:1n-5C; C20:3n-7c; at group B the fatty acids that explained 51.52% of the group similarity, in decreasing order of importance, were: DHA; C17:1n-8c; EPA; C16:0; C16:1n-7t; C17:0; C18:0; C15:1n-5c; ARA; C20:1n-9c; C22:1n-9c; C15:0; C22:2n-6c; ALA; at group C the fatty acids that explained 70.58% of the group similarity were, in decreasing order of importance: DHA; C16:0; EPA; ARA; C17:1n-8c; C20:1n-9c; C18:0; C16:1n-7t; C18:1n-9t; C17:0; C22:0; C22:2n-6c; C22:1n-9c; at group D the fatty acids that explained 77.82% of the similarity within the group were, in decreasing order of importance: DHA; C16:0; EPA; C18:0; ARA; C22:1n-9c; C16:1n-7t; C17:1n-8c; C18:1n-9t; C20:1n-9c; C22:0; C22:2n-6c (Table 7). In general, the main fatty acids that most contributed for the similarities within each group were DHA, C16:0; C17:1n-8c and EPA.

Table 7. Results of SIMPER analyses of fatty acid abundance showing average similarity among the species inside each group according to non-metric multidimensional scaling (n-MDS) analysis.

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
		C18:0	0.00	3.35	15.34	5.02	83.28
		C15:1n-5c	0.00	3.00	7.31	4.49	87.77
		C20:3n-7c	0.01	2.11	0.58	3.17	90.94
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
		C17:0	0.05	2.37	1.39	4.60	72.29
		C18:0	0.04	1.85	1.76	3.58	75.88
		C15:1n-5c	0.04	1.65	1.71	3.21	79.09
		C20:4n-6c ARA	0.07	1.47	1.06	2.86	81.95
		C20:1n-9c	0.04	0.99	1.10	1.93	83.88
		C22:1n-9c	0.04	0.94	1.15	1.82	85.70
		C15:0	0.02	0.89	1.18	1.73	87.43
		C22:2n-6c	0.03	0.88	0.92	1.71	89.14
		C18:3n-3c ALA	0.02	0.84	1.73	1.64	90.78

Table 7. Results of SIMPER analyses of fatty acid abundance showing average similarity among the species inside each group according to non-metric multidimensional scaling (n-MDS) analysis (cont.).

Group	Average Similarity	Fatty Acids	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
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
		C20:1n-9c	0.44	2.48	3.40	3.51	77.57
		C18:0	0.58	2.42	3.03	3.43	81.01
		C16:1n-7t	0.40	1.29	1.11	1.83	82.84
		C18:1n-9t	0.50	1.22	2.41	1.73	84.57
		C17:0	0.33	1.20	3.46	1.69	86.26
		C22:0	0.33	1.11	3.24	1.57	87.83
		C22:2n-6c	0.31	1.05	2.90	1.48	89.32
		C22:1n-9c	0.29	0.95	0.89	1.35	90.67
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
		C22:1n-9c	1.63	2.91	3.07	3.74	78.44
		C16:1n-7t	1.65	2.10	2.64	2.70	81.14
		C17:1n-8c	0.85	1.76	3.09	2.26	83.39
		C18:1n-9t	0.84	1.64	3.49	2.10	85.50
		C20:1n-9c	1.87	1.46	9.20	1.88	87.38
		C22:0	0.74	1.46	6.52	1.87	89.25
		C22:2n-6c	1.12	1.42	1.68	1.83	91.08

In what concerns the dissimilarities between groups, the fatty acids that contributed, in decreased order of importance, for i) 82.28% of the dissimilarity among the groups A/B were DHA; C17:1n-8c; EPA; C16:0; C16:1n-7t; ARA; C17:0; C15:1n-5c; C18:0; C20:1n-9c; C22:1n-9c; C22:2n-6c; C20:2n-6c; C15:0; C18:1n-9t; C18:1n-9c, ii) 98.73% of the dissimilarity among the groups A/C were DHA; EPA; C16:0; C20:0; ARA; C17:1n-8c; C18:0; C18:1n-9t; C20:1n-9c; C16:1n-7t; C20:2n-6c; C22:0; C17:0; C22:2n-6c; C22:1n-9c; C18:1n-9c, iii) 99.53% of the dissimilarity among the groups A/D were DHA; EPA; C16:0; C18:0; ARA; C22:1n-9c; C20:1n-9c; C16:1n-7t; C22:2n-6t; C17:1n-8c; C18:1n-9t; C22:0; C20:2n-6c; ALA; C23:0, iv) 84.37% of the dissimilarity among the groups B/C were DHA; EPA; C16:0; C20:0; ARA; C18:0; C18:1n-9t; C17:1n-8c; C20:1n-9c; C20:2n-6c; C22:0; C16:1n-7t; C22:2n-6c; C22:1n-9c; C17:0; C18:1n-9c, v) 93.71% of the dissimilarity among the groups B/D were DHA; EPA;C16:0; C18:0;ARA; C22:1n-9c; C20:1n-9c; C16:1n-7t; C22:2n-6c; C18:1n-9t; C22:0; C17:1n-8c; C20:2n-6c; ALA; C23:0 and vi) 51.03% of the dissimilarity among the groups C/D were explained by DHA; EPA;C16:0; C22:1n-9c; C20:1n-9c; C16:1n-7t; C18:0; C20:0; ARA; C22:2n-6c; C20:2n-6c; C18:1n-9t; C18:3n-3c ALA; C22:0; C20:3n-7c;C18:2n-6t (Table 8). Almost all dissimilarities between groups were mainly explained by DHA and EPA, only the dissimilarity between A/B was mainly explained by DHA and C16:0.

Table 8. Results of SIMPER analyses of fatty acid abundance showing average dissimilarity between sample groups according to non-metric multidimensional scaling (n-MDS) analysis.

Group	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
		C20:4n-6c ARA	0.01	0.07	3.29	1.14	4.00	65.12
		C17:0	0.01	0.05	3.25	1.35	3.95	69.07
		C15:1n-5c	0.00	0.04	2.56	1.49	3.12	72.18
		C18:0	0.00	0.04	2.48	2.04	3.01	75.20
		C20:1n-9c	0.00	0.04	2.35	1.12	2.86	78.06
		C22:1n-9c	0.00	0.04	2.19	1.42	2.66	80.72
		C22:2n-6c	0.00	0.03	2.11	0.84	2.57	83.29
		C20:2n-6c	0.00	0.05	1.87	0.80	2.27	85.56
		C15:0	0.00	0.02	1.54	1.10	1.88	87.44
		C18:1n-9t	0.00	0.02	1.13	1.56	1.38	88.81
		C18:1n-9c	0.00	0.02	1.06	1.61	1.28	90.10
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
		C17:1n-8c	0.03	0.58	3.88	2.19	3.93	66.93
		C18:0	0.00	0.58	3.57	3.17	3.62	70.55
		C18:1n-9t	0.00	0.50	2.98	1.31	3.02	73.56
		C20:1n-9c	0.00	0.44	2.98	3.17	3.02	76.58
		C16:1n-7t	0.01	0.40	2.37	1.72	2.40	78.98
		C20:2n-6c	0.00	0.28	2.22	0.88	2.25	81.24
		C22:0	0.00	0.33	2.16	1.61	2.19	83.42
		C17:0	0.01	0.33	1.97	2.17	1.99	85.42
		C22:2n-6c	0.00	0.31	1.97	1.67	1.99	87.41
		C22:1n-9c	0.00	0.29	1.96	1.57	1.98	89.39
		C18:1n-9c	0.00	0.25	1.53	1.75	1.55	90.94
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
		C22:1n-9c	0.00	1.63	3.87	3.67	3.89	70.12
		C20:1n-9c	0.00	1.87	3.78	0.99	3.80	73.90
		C16:1n-7t	0.01	1.65	3.65	1.87	3.67	77.56
		C22:2n-6t	0.00	1.12	2.57	1.53	2.58	80.15
		C17:1n-8c	0.03	0.85	2.11	2.52	2.12	82.26
		C18:1n-9t	0.00	0.84	2.06	2.94	2.07	84.33
		C22:0	0.00	0.74	1.84	3.08	1.85	86.18
		C20:2n-6c	0.00	0.74	1.60	1.38	1.61	87.79
		C18:3n-3c ALA	0.00	0.69	1.52	2.32	1.52	89.31
		C23:0	0.00	0.52	1.27	4.11	1.27	90.59
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
		C18:0	0.04	0.58	3.08	2.72	3.66	67.47
		C18:1n-9t	0.02	0.50	2.68	1.29	3.17	70.64
		C17:1n-8c	0.18	0.58	2.64	1.62	3.13	73.77
		C20:1n-9c	0.04	0.44	2.51	2.80	2.97	76.74
		C20:2n-6c	0.05	0.28	1.95	0.93	2.31	79.05
		C22:0	0.01	0.33	1.91	1.56	2.27	81.32
		C16:1n-7t	0.06	0.40	1.88	1.47	2.23	83.55
		C22:2n-6c	0.03	0.31	1.65	1.52	1.96	85.51
		C22:1n-9c	0.04	0.29	1.61	1.48	1.91	87.42
		C17:0	0.05	0.33	1.58	1.78	1.87	89.29
		C18:1n-9c	0.02	0.25	1.34	1.65	1.59	90.88

Table 8. Results of SIMPER analyses of fatty acid abundance showing average dissimilarity between sample groups according to non-metric multidimensional scaling (n-MDS) analysis (cont.).

Group	Average Dissimilarity	Fatty Acids	Av. Abund		Av. Diss	Diss/SD	Contrib%	Cum.%
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
		C22:1n-9c	0.04	1.63	3.66	3.58	3.90	70.40
		C20:1n-9c	0.04	1.87	3.58	0.99	3.82	74.23
		C16:1n-7t	0.06	1.65	3.41	1.81	3.64	77.86
		C22:2n-6c	0.03	1.12	2.43	1.53	2.59	80.46
		C18:1n-9t	0.02	0.84	1.96	3.00	2.09	82.55
		C22:0	0.01	0.74	1.75	3.11	1.86	84.41
		C17:1n-8c	0.18	0.85	1.67	2.09	1.78	86.20
		C20:2n-6c	0.05	0.74	1.51	1.44	1.61	87.81
		C18:3n-3c ALA	0.02	0.69	1.45	2.29	1.54	89.35
		C23:0	0.01	0.52	1.21	4.13	1.29	90.64
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
		C16:1n-7t	0.40	1.65	2.04	1.19	4.00	67.80
		C18:0	0.58	1.67	2.02	1.83	3.97	71.77
		C20:0	1.00	0.41	1.77	1.09	3.47	75.24
		C20:4n-6c ARA	0.91	1.74	1.45	1.37	2.84	78.08
		C22:2n-6c	0.31	1.12	1.45	1.18	2.84	80.92
		C20:2n-6c	0.28	0.74	1.01	1.37	1.99	82.91
		C18:1n-9t	0.50	0.84	0.89	1.51	1.74	84.64
		C18:3n-3c ALA	0.16	0.69	0.85	1.42	1.66	86.30
		C22:0	0.33	0.74	0.77	1.46	1.52	87.82
		C20:3n-7c	0.02	0.43	0.74	1.07	1.45	89.26
		C18:2n-6t	0.00	0.45	0.63	0.55	1.24	90.50

C. edule showed higher FA abundance in winter than in summer at the Mondego estuary (1.544 µg/g and 0.897 µg/g of total FA in winter and in summer, respectively) and at the Ria Formosa lagoon (40.541 µg/g and 16.666 µg/g of total FA in winter in summer, correspondingly), but it was not considered significant at the estuarine system, as the samples from both seasons were aggregated in the same n-MDS group because of the similarities between them. Indeed, *C. edule* was the bivalve species with the greatest FA profile abundance in both seasons at the Ria Formosa lagoon. The FA profiles of *C. edule* in both studied areas and seasons were constituted by a considerable portion of HUFAs (37.111% and 39.688% of the total FA in winter and in summer in the Mondego estuary, correspondingly; 51.920% and 51.050% of the total FA in winter and in summer in Ria Formosa, respectively), namely DHA, EPA and AA, followed by the contribution of SFAs (27.396% and 27.871% of the total FA in winter and in summer in the Mondego estuary, respectively; 26.625% and 24.787% of the total FA in winter and in summer in Ria Formosa, correspondingly), mainly C14:0, C16:0, C17:0, C22:0 and C18:0, the contribution of MUFAs (29.339% and 24.303% of the total FA in winter and in summer in the Mondego estuary, respectively; 12.363% and 17.587% of the total FA in winter and in summer in Ria Formosa, correspondingly), that was mainly represented by

C15:1n-5c, C16:1n-7t, C17:1n-8c, C20:1n-9c and C22:1n-9c, and the contribution of PUFAs (6.153% and 8.138% of the total FA in winter and in summer in the Mondego estuary, respectively; 9.092% and 6.576% of the total FA in winter and in summer in Ria Formosa, correspondingly), that was principally composed by C22:2n-6c, C20:2n-6c, ALA and LA.

C. gigas sampled only in the Ria Formosa lagoon (0.891 µg/g and 0.112 µg/g of total FA in winter in summer, respectively) showed higher FA abundance in winter than in summer. The samples collected in these two seasons were segregated in two distinct n-MDS groups due to the dissimilarities between them. The FA profile of *C. gigas* in Ria Formosa was composed by a percentage of HUFAs higher in winter (34.231% of the total FA) than in summer (29.464% of the total FA) and was composed by DHA, EPA, and AA. The contribution and diversity of PUFAs was higher in winter (15.825% of the total FA), when C18:2n-6t, C20:2n-6c and C22:2n-6c were found, than in summer (1.786% of the total FA), when only C18:2n-6t was detected. The contribution of MUFAs (37.823% and 51.786% of the total FA in winter and in summer, respectively) was greater than the contribution of SFAs, PUFAs and HUFAs in both seasons and was mainly composed by C15:1n-5c, C16:1n-7t and C17:1n-8c. Despite having a higher contribution of SFAs in summer (16.964% of the total FA), the FA content in this season was less diverse than in winter (12.121% of the total FA) and was primarily constituted by C16:0, C17:0 and C18:0.

M. galloprovincialis S collected at the Mondego estuary showed significantly higher FA content in winter (214.037 µg/g) than in summer (55.746 µg/g) and presented a distinct FA content compared to the other species that may explain why these samples were not included in any of the groups of n-MDS. *M. galloprovincialis* B showed significant differences in the FA profiles in Mondego estuary (11.683 µg/g and 2.038 µg/g in winter and in summer, respectively), presenting higher FA concentrations in winter, while in Ria Formosa (4.186µg/g and 1.853 µg/g in winter and in summer, correspondingly), and despite presenting higher FA content, the abundance in winter was not considered significantly different when compared with summer. *M. galloprovincialis* B, sampled in winter at the Mondego estuary (11.683 µg/g), also demonstrated to have higher FA abundance than in winter at Ria Formosa (4.186µg/g). *M. galloprovincialis* small sampled at the Mondego estuary presented higher FA concentrations (214.037 µg/g and 55.746 µg/g in winter and in summer, respectively) than big size organisms (11.683 µg/g and 2.038 µg/g in winter and in summer, correspondingly) in both seasons. *M. galloprovincialis* was the bivalve species with the

greatest FA content in the Mondego estuary and with the second greatest content in FA in Ria Formosa, after *C. edule*. The major contribution for the total FA content in the edible tissues of both sizes of *M. galloprovincialis*, in both seasons and studied areas, were HUFAs (49.125% and 47.588% of the total FA in winter and in summer of small-size organisms from the estuarine system, respectively; 53.796% and 42.296% of the total FA in winter and in summer of big-size organisms from the Mondego estuary, correspondingly; 48.232% and 36.589% of the total FA in winter and in summer of big-size bivalves from Ria Formosa, respectively), that included DHA, EPA and AA. It was followed by the contributions of SFAs (24.175% and 14.610% of the total FA in winter and in summer of small-size individuals from the Mondego estuary, respectively; 19.567% and 23.847% of the total FA in winter and in summer of big-size organisms from the estuarine system, correspondingly; 16.961% and 19.914% of the total FA in winter and in summer of big-size individuals from Ria Formosa, respectively), including C14:0, C16:0, C17:0 and C18:0. MUFA content was higher in small organisms than in big individuals from the Mondego estuary (22.068% of the total FA in winter and 26.398% in summer of small size individuals from the Mondego estuary; 17.136% of the total FA in winter and 22.080% in summer of big size individuals from the estuarine system) whereas organisms from Ria Formosa showed the highest MUFA content (24.964% of the total FA in winter and 39.018% in summer of big size individuals from Ria Formosa). The most abundant MUFAs were C20:1n-9c, C22:1n-9c, C16:1n-7t and C17:1n-8c. The contribution of PUFAs was higher in summer of both sizes than in winter, at the Mondego estuary (4.632% and 11.404% of the total FA in winter and in summer of small size individuals, respectively, and 9.501% and 11.76% of the total FA in winter and in summer of big size organisms, correspondingly, from the Mondego estuary), while it was higher in big size organisms sampled in winter, at the Ria Formosa lagoon (9.842% of the total FA in winter and 4.479% in summer of big size individuals), and it was mainly composed by LA, C20:2n-6c and C22:2n-6c.

R. decussatus showed higher FA abundance in both seasons at the Mondego estuary (34.613 µg/g and 35.824 µg/g in winter and in summer, respectively) than at Ria Formosa (0.785 µg/g and 0.755 µg/g in winter and in summer, correspondingly), being the second bivalve species with the greatest FA composition in the estuarine system, after *M. galloprovincialis* S. In the lagoon system, this species showed significantly higher FA concentrations in winter rather than in summer. The FA content of *R. decussatus* in the Mondego estuary were constituted by a substantial portion of HUFAs in both seasons, while in Ria Formosa the composition and diversity of these FAs was lower

(53.422 and 53.690% of the total FA in winter and in summer, respectively, at the estuarine system; 16.815% and 19.735% of the total FA in winter and in summer, correspondingly, at the lagoon system), even being surpassed by the large abundance of SFAs in winter (64.713% of the total FA) and the large abundance of MUFAs in summer (47.550% of the total FA). The main HUFAs found were DHA, EPA and AA. The contribution of PUFAs (2.670% and 4.949% of total FA in winter and in summer at the Mondego estuary, respectively; 1.019% in winter and 4.503% in summer at Ria Formosa) was principally due to ALA, LA and C22:2n-6c. The main SFAs contributors for their profile were C16:0, C18:0, C17:0 and C22:0 (26.172% of the total FA in winter and 25.223% in summer at the estuarine system; 64.713% of the total FA in winter and 28.212% in summer at the lagoon system). The contribution of MUFAs (17.736% of total FA in winter and 16.127% in summer at the Mondego estuary; 17.452% in winter and 47.550% in summer at Ria Formosa) was principally due to the abundances of C15:1n-5c, C16:1n-7t, C17:1n-8c, C20:1n-9c and C22:1n-9c.

S. plana collected only at the Mondego estuary had a significant higher FA content in winter than in summer (17.640 µg/g and 0.669 µg/g in winter and in summer, respectively). The FA profile of *S. plana* from the estuarine system was composed by a substantial portion of HUFAs in both seasons (37.460% and 44.544% of the total FA in winter and in summer, respectively), including the essential fatty acids DHA, EPA and AA. The contribution of SFAs (39.598% and 23.318% of the total FA in winter and in summer, respectively, in the Mondego estuary) was mainly composed by C16:0, C17:0 and C18:0 in both seasons. It was greater in winter than the contribution of MUFAs (18.571% and 27.653% of the total FA in winter and in summer, respectively, in the estuarine system) and lower and less diverse in summer than the contribution of MUFAs, which was primarily composed by C16:1n-7t, C17:1n-8c, C18:1n-9t and C20:1n-9c. The contribution of PUFAs (4.371% and 4.484% of the total FA in winter and in summer, respectively, in the Mondego estuary) was similar in both seasons and was mainly composed by LA and ALA.

S. marginatus showed higher FA abundance in winter than in summer (0.525 µg/g in winter and 0.081 µg/g in summer) at the Mondego estuary and did not show significant differences between the two studied areas, in any of the seasons. The FA content of *S. marginatus* in Ria Formosa (0.407 µg/g and 0.106 µg/g in winter and in summer, respectively) was constituted mainly by HUFAs (18.857% and 19.753% of the total FA in winter and in summer, correspondingly, at the Mondego estuary; 38.821% and 33.962% of the total FA in winter and in summer, respectively, at the lagoon system)

and PUFAs (4.381% and 13.580% of the total FA in winter and in summer, correspondingly, at the Mondego estuary; 4.423% and 11.321% in winter and in summer at the Ria Formosa lagoon, respectively), while at the Mondego estuary the main contributors for the FA content were MUFAs (49.5249% of the total FA in winter and 43.210% in summer at the estuarine system; 34.644% of the total FA in winter and 31.132% in summer at the lagoon system). The main HUFAs found in this species was DHA, not presenting AA concentrations at the Mondego estuary and EPA concentrations in the summer samples of both studied areas. In terms of PUFA content, ALA, LA and C22:2n-6c were the main contributors. The MUFAs found in higher abundances were C17:1n-8c, C16:1n-7t and C15:1n-5c. The SFA contribution (27.238% of the total FA in winter and 23.457% in summer at the Mondego estuary; 22.113% of the total FA in winter and 23.585% in summer at Ria Formosa) was composed principally by C16:0 and C17:0, being less diverse in summer samples from both studied areas.

3.2 Total Protein Content

Total protein content in bivalve tissue was obtained for each species in both study areas and seasons. Kruskal-Wallis H test ($H = 93.758$, $p < 0.05$) showed significant differences between the groups' distributions considering the total protein content of different bivalve species in both seasons and studied areas. The null hypothesis b) the total protein content is equal between species and does not show spatial and seasonal variations was rejected. The pairwise two-tailed Mann-Whitney U tests performed estimated which groups had significant different distributions of total protein content ($p \leq 0.05$) (Appendix I). In general, all bivalve species from the Mondego estuary and the Ria Formosa lagoon demonstrated higher protein contents in winter (Figure 4).

C. edule appeared to have significantly higher protein content ($2.242 < Z < 2.722$; $N = 12$; $0.004 < p < 0.026$) in winter seasons at both studied areas (2643.248 ± 240.638 µg/g in winter and 1278.878 ± 111.900 µg/g in summer at the Mondego estuary; 3086.836 ± 133.234 µg/g in winter and 1660.407 ± 156.550 µg/g in summer at Ria Formosa). Within each season, *C. edule* specimens did not present significant differences in protein concentration between both sites, the Mondego estuary and the Ria Formosa lagoon ($1.281 < Z < 1.601$, $N = 12$, $0.132 < p < 0.240$). In winter, at the estuarine system, *C. edule* showed a lower content in protein than *M. galloprovincialis* B, although it presented a higher content than *R. decussatus* ($-2.562 < Z < 2.082$, $N =$

12, 0.009 < p < 0.041) and also higher total protein value than all bivalve species from Ria Formosa in summer (2.542 < Z < 2.882, N = 12, 0.002 < p < 0.026). The species that did not demonstrate statistical differences with *C. edule* sampled in winter at the Mondego estuary were i) *M. galloprovincialis* S, *R. decussatus*, *S. plana* and *S. marginatus* collected in both seasons at the estuarine system; ii) *M. galloprovincialis* B sampled in summer at the Mondego estuary; iii) *C. edule*, *C. gigas*, *M. galloprovincialis* B and *R. decussatus* collected in winter at the Ria Formosa lagoon (Figure 4 and Appendix I). At the Mondego estuary, *C. edule* sampled in summer presented a i) lower protein content than *M. galloprovincialis* S, *M. galloprovincialis* B and *S. plana* sampled in both seasons, and *R. decussatus* and *S. marginatus* sampled in winter at the Mondego estuary (-2.882 < Z < -2.722, N = 12, 0.002 < p < 0.004); ii) lower protein content than *C. gigas*, *M. galloprovincialis* B, *R. decussatus* and *S. marginatus* sampled in winter at Ria Formosa and higher protein content than *M. galloprovincialis* B and *S. marginatus* sampled in summer at the lagoon system (-2.882 < Z < 2.562, N = 12, 0.002 < p < 0.041). The species that did not demonstrate statistical differences with *C. edule* sampled in summer at the estuarine system were i) *R. decussatus* and *S. marginatus* collected in summer at the Mondego estuary; ii) *S. marginatus* sampled in winter at the lagoon system; iii) *C. edule*, *C. gigas* and *R. decussatus* collected in summer at the Ria Formosa lagoon (Figure 4 and Appendix I). At Ria Formosa lagoon, in winter, *C. edule* showed a i) lower protein content than *M. galloprovincialis* B sampled in winter, at the Mondego estuary, and higher protein content than *R. decussatus* and *S. marginatus* collected in both seasons at the estuarine system (-2.082 < Z < 2.882, N = 12, 0.002 < p < 0.041; ii) higher protein content than *S. marginatus* sampled in winter and all species sample in summer, at the Ria Formosa lagoon (2.562 < Z < 2.882, N = 12, 0.002 < p < 0.014). The species that did not demonstrate statistical differences with *C. edule* sampled in winter at the Ria Formosa lagoon were i) *C. edule* and *S. marginatus* sampled in winter at the Mondego estuary; ii) *M. galloprovincialis* S and *S. plana* in both seasons at the estuarine system; iii) *M. galloprovincialis* B collected in summer at the Mondego estuary; iv) *C. gigas*, *M. galloprovincialis* B and *R. decussatus* sampled in winter at the Ria Formosa lagoon (Figure 4 and Appendix I). *C. edule* showed the highest protein content of all bivalve species collected in summer at the Ria Formosa lagoon (0.642 < Z < 2.722, N = 12, 0.002 < p < 0.593). At the lagoon system, *C. gigas*, *M. galloprovincialis* B and *R. decussatus* sampled in winter showed higher amount in protein than *C. edule* sampled in summer (2.562 < Z < 2.882, N = 12, p = 0.002). Comparing with the species collected at the Mondego estuary, the cockle, sampled in summer at the lagoon system, presented

lower protein content than *M. galloprovincialis* S, *M. galloprovincialis* B and *S. plana* sampled in both seasons and *S. marginatus* collected in winter ($-2.882 < Z < -2.242$, $N = 12$, $0.002 < p < 0.026$). The species that did not demonstrate statistical differences with *C. edule* sampled in summer at the Ria Formosa lagoon were i) *C. edule* and *S. marginatus* collected in summer at the estuarine system; ii) *R. decussatus* sampled in both seasons at the Mondego estuary; iii) *S. marginatus* collected in winter at the lagoon system; iv) *C. gigas* and *R. decussatus* sampled in summer at the Ria Formosa lagoon (Figure 4 and Appendix I).

C. gigas, sampled in Ria Formosa, showed a significant higher protein content ($Z = 2.722$, $N = 12$, $p = 0.004$) in winter (4626.815 ± 813.828 µg/g) than in summer (1275.645 ± 301.732 µg/g). Indeed, *C. gigas* collected in winter at Ria Formosa presented the highest protein content in this studied area and season, and showed significantly higher protein values than *S. marginatus* collected in both seasons, and *C. edule*, *M. galloprovincialis* B and *R. decussatus* sampled in summer ($2.722 < Z < 2.882$, $N = 12$, $0.001 < p < 0.002$). *C. gigas,* sampled in winter, also showed higher values than the following species collected at the Mondego estuary: *R. decussatus* and *S. marginatus* collected in both seasons, *C. edule* and *M. galloprovincialis* S and B collected in summer ($2.082 < Z < 2.882$, $N = 12$, $0.002 < p < 0.041$). The species that did not demonstrate statistical differences with *C. gigas* sampled in winter at Ria Formosa were i) *C. edule*, *M. galloprovincialis* B and *R. decussatus* collected in winter at the lagoon system; ii) *C. edule*, *M. galloprovincialis* S and B sampled in winter and *S. plana* collected in both seasons at the Mondego estuary (Figure 4 and Appendix I). In summer, at the Ria Formosa lagoon, *C. gigas* presented the third highest protein content of the species here collected, and showed lower protein content than *C. edule*, *M. galloprovincialis* B and *R. decussatus* collected in winter ($-2.562 < Z < -2.082$, $N = 12$, $0.004 < p < 0.013$). Comparing with the species collected at the Mondego estuary, *C. gigas,* sampled in summer at the Ria Formosa lagoon, presented lower protein content than *C. edule* and *S. marginatus* collected in winter, and *M. galloprovincialis* S and B and *S. plana* sampled at both seasons ($-2.722 < Z < -2.082$, $N = 12$, $0.002 < p < 0.041$). The species that did not demonstrate statistical differences with *C. gigas*, collected in summer at the lagoon system, were i) *C. edule*, *M. galloprovincialis* B and *R. decussatus* sampled in summer and *S. marginatus* collected at both seasons at Ria Formosa; ii) *C. edule* e *S. marginatus* sampled in summer and *R. decussatus* collected at both seasons in the Mondego estuary (Figure 4 and Appendix I).

M. galloprovincialis S collected at the Mondego estuary did not show significant differences ($Z = 1.1121$, $N = 12$, $p = 0.310$) between protein content from winter and summer (3197.617 ± 332.372 µg/g and 2625.111 ± 248.431 µg/g, respectively), while *M. galloprovincialis* B showed significant higher protein content ($2.402 < Z < 2.882$, $N = 12$, $0.002 < p < 0.015$) in winter for both studied areas (4076.924 ± 420.075 µg/g in winter and 2634.055 ± 358.418 µg/g in summer at the Mondego estuary; 3293.882 ± 395.306 µg/g in winter and 835.631 ± 124.383 µg/g in summer at Ria Formosa). Within each season, small-size and big-size specimens of *M. galloprovincialis* collected at the Mondego estuary did not show a significant difference between each size ($0.641 < Z < 1.121$, $N = 12$, $0.310 < p < 0.589$), even with big-size organisms showing higher content than the small-size at both seasons. In winter, *M. galloprovincialis* B did not presented significant different protein content between Mondego estuary and Ria Formosa ($Z = 1.281$, $N = 12$, $p = 0.240$), whereas in summer, the big-size specimens showed a significant higher protein content at the Mondego estuary ($Z = 2.882$, $N = 12$, $p = 0.002$).

At the Mondego estuary, in winter, *M. galloprovincialis* S presented the second highest protein content of the species collected, and significantly higher protein content than *R. decussatus* collected at both seasons, and *C. edule* and *S. marginatus* collected in summer ($2.403 < Z < 2.882$, $N = 12$, $0.002 < p < 0.011$). Comparing with the species sampled in Ria Formosa, *M. galloprovincialis* S presented higher protein content that *S. marginatus* sampled at both seasons and all bivalve species collected in summer ($2.082 < Z < 2.882$, $N = 12$, $0.002 < p < 0.041$). The species that did not demonstrate statistical differences with *M. galloprovincialis* S collected in winter at the Mondego estuary were i) *C. edule* and *S. marginatus* sampled in winter, *M. galloprovincialis* S collected in summer and *M. galloprovincialis* B and *S. plana* sampled in both seasons at the estuarine system; ii) *C. edule*, *C. gigas*, *M. galloprovincialis* B and *R. decussatus* collected in winter at the Ria Formosa lagoon (Figure 4 and Appendix I). In summer, at the Mondego estuary, *M. galloprovincialis* S showed a higher protein content than *C. edule* and *R. decussatus*. Comparing with the species collected in Ria Formosa, *M. galloprovincialis* S, sampled in summer at the Mondego estuary, presented higher protein content than *C. edule*, *C. gigas*, *R. decussatus* and *S. marginatus* collected in summer, and lower protein content than *C. gigas* sampled in winter ($-2.082 < Z < 2.882$, $N = 12$, $0.002 < p < 0.041$). The species that did not demonstrate statistical differences with *M. galloprovincialis* S in summer at the Mondego estuary were i) *C. edule, M. galloprovincialis* S and *R. decussatus* sampled in winter, *M. galloprovincialis* B collected in summer, and *S. plana* and *S. marginatus* sampled in both seasons at the estuarine system; ii) *C. edule*, *M.*

galloprovincialis B and *R. decussatus* collected in winter at the Ria Formosa lagoon (Figure 4 and Appendix I).

At the Mondego estuary, in winter, *M. galloprovincialis* B presented the highest protein content that was also significantly higher than i) *C. edule*, *R. decussatus*, *S. plana* and *S. marginatus* collected in both seasons at the estuarine system; ii) *C. edule, R. decussatus* and *S. marginatus* sampled at both seasons in Ria Formosa; iii) *C. gigas* collected in summer at the Ria Formosa lagoon ($2.082 < Z < 2.882$, $N = 12$, $0.002 < p < 0.041$). The species that did not demonstrate statistical differences with *M. galloprovincialis* B in winter at the Mondego estuary were *M. galloprovincialis* S and *C. gigas* sampled in winter at the estuarine system and at the lagoon system, respectively (Figure 4 and Appendix I). At the Mondego estuary, in summer, *M. galloprovincialis* B showed a i) higher protein content than *R. decussatus* collected at both seasons, *C. edule* and *S. marginatus* sampled in summer at the estuarine system; ii) higher protein content than *S. marginatus* collected in winter and all bivalve species sampled in summer at the Ria Formosa lagoon; iii) lower protein content than *C. gigas* collected in winter at Ria Formosa ($-2.242 < Z < 2.882$, $N = 12$, $0.002 < p < 0.041$). The species that did not demonstrate statistical differences with *M. galloprovincialis* B in summer at the Mondego estuary were i) *C. edule* and *R. decussatus* sampled in winter at the estuarine system; ii) *M. galloprovincialis* S, *S. plana* and *S. marginatus* collected in both seasons at the Mondego estuary; iii) *C. edule*, *M. galloprovincialis* B and *R. decussatus* sampled in winter at the Ria Formosa lagoon (Figure 4 and Appendix I). In Ria Formosa, *M. galloprovincialis* B collected in winter presented a higher protein content than *S. marginatus* in winter and all species sampled in summer ($2.082 < Z < 2.882$, $N = 12$, $0.002 < p < 0.041$). Comparing with the species collected at the Mondego estuary, *M. galloprovincialis* B, sampled in winter at Ria Formosa, showed a higher protein content than i) *R. decussatus* sampled at both seasons and *C. edule* and *S. marginatus* collected in summer ($2.243 < Z < 2.882$, $N = 12$, $0.001 < p < 0.029$). The species that did not demonstrate statistical differences with *M. galloprovincialis* B in winter in Ria Formosa were i) *C. edule*, *C. gigas* and *R. decussatus* sampled in winter at the lagoon system; ii) *C. edule* and *S. marginatus* collected in winter at the Mondego estuary; iii) *M. galloprovincialis* S and *S. plana* sampled in both seasons at the estuarine system; iv) *M. galloprovincialis* B collected in summer at the Mondego estuary (Figure 4 and Appendix I). In Ria Formosa, *M. galloprovincialis* B collected in summer showed a lower protein content than all species sampled in both seasons at the Mondego estuary, all species collected in winter at the Ria Formosa lagoon and *C. edule* and *R. decussatus* sampled

in summer in the lagoon system (-2.882 < Z < -2.082, N = 12, 0.002 < p < 0.041). The species that did not demonstrate statistical differences with *M. galloprovincialis* B, in summer at the Ria Formosa lagoon, were *C. gigas* and *S. marginatus* collected in summer at the lagoon system (Figure 4 and Appendix I).

R. decussatus showed significant higher protein content (Z = 2.882, N = 12, p = 0.002) in winter at the Ria Formosa lagoon, while at the Mondego estuary, the protein content in winter samples was not significantly higher (Z = 1.121, N = 12, p = 0.310) than the content found in summer (2016.670 ± 144.402 µg/g in winter and 1785.439 ± 256.150 µg/g in summer at the Mondego estuary; 2977.943 ± 145.571 µg/g in winter and 1491.844 ± 149.149 µg/g in summer in Ria Formosa). In winter, *R. decussatus* exhibited higher protein contents in Ria Formosa than in the Mondego estuary (Z =2.562, N = 12, p =0.009), while in summer, it showed no significant differences in protein content between the study areas (Z = 0.801, N = 12, p = 0.485). At the Mondego estuary, in winter, *R. decussatus* presented a i) lower protein content than *C. edule*, *M. galloprovincialis* S and *S. plana* sampled in winter and *M. galloprovincialis* B collected at both seasons at the Mondego estuary; ii) lower protein value than *C. edule, C. gigas* and *M. galloprovincialis* B sampled in winter at the Ria Formosa lagoon; iii) higher protein content than *C. edule* collected in summer at the estuarine system; iv) higher protein value than *M. galloprovincialis* B and *S. marginatus* sampled in summer at the lagoon system (-2.722 < Z < 2.882, N = 12, 0.002 < p < 0.041). The species that did not demonstrate statistical differences with *R. decussatus* collected in winter at the Mondego estuary were i) *C. edule* sampled in winter at the estuarine system; ii) *M. galloprovincialis* S and B and *S. plana* collected in summer at the estuary; iii) *S. marginatus* at both seasons at the Mondego estuary; iv) *C. edule* and *C. gigas* sampled in summer and *S. marginatus* collected in winter at the Ria Formosa lagoon (Figure 4 and Appendix I). *R. decussatus* collected in summer at the Mondego estuary presented a protein content i) lower than *M. galloprovincialis* S and B sampled in both seasons and *S. plana* collected in winter at the estuarine system; ii) lower than *C. edule*, *C. gigas* and *M. galloprovincialis* B collected in winter at the Ria Formosa lagoon: iii) higher that *M. galloprovincialis* B and *S. marginatus* sampled in summer at the lagoon system (-2.722 < Z < 2.882, N = 12, 0.002 < p < 0.041). The species that did not demonstrate statistical differences with *R. decussatus* collected in summer at the Mondego estuary were i) *C. edule* and *S. marginatus* sampled in both seasons, *R. decussatus* in winter and *S. plana* in summer at the estuarine system; ii) *C. edule*, *C. gigas* and *R. decussatus* collected in summer and *S. marginatus* in winter at the Ria Formosa lagoon (Figure 4 and Appendix I). *R.*

decussatus collected in winter at the Ria Formosa lagoon presented a protein content i) lower than *M. galloprovincialis* B sampled in winter at the Mondego estuary; ii) higher than *C. edule* and *S. marginatus* collected in summer at the estuarine system; iii) higher than *S. marginatus* sampled in winter and all species sampled in summer at the Ria Formosa lagoon ($-2.882 < Z < 2.882$, $N = 12$, $0.002 < p < 0.026$). The species that did not demonstrate statistical differences with *R. decussatus* collected in winter at Ria Formosa were i) *C. edule* and *S. marginatus* sampled in winter, *M. galloprovincialis* B collected in summer, *M. galloprovincialis* S and *S. plana* sampled in both seasons at the Mondego estuary; ii) *C. edule*, *C. gigas*, *M. galloprovincialis* B collected in winter at the lagoon system (Figure 4 and Appendix I). Comparing with other species from Ria Formosa, the protein content of *R. decussatus* sampled in summer in Ria Formosa presented the second highest protein content of all species in this area and season. Under this sampling conditions, *R. decussatus* showed a protein content lower than i) all species sampled in winter at the Mondego estuary, ii) *M. galloprovincialis* S and B and *S. plana* collected in summer at the Mondego estuary and iii) all bivalve species sampled in winter in Ria Formosa ($-2.882 < Z < -2.242$, $N = 12$, $0.001 < p < 0.029$). On the other hand, *R. decussatus* presented a higher protein content than *M. galloprovincialis* B and *S. marginatus* collected in summer in Ria Formosa ($2.401 < Z < 2.562$, $N = 12$, $0.013 < p < 0.015$). The species that did not demonstrate statistical differences with *R. decussatus* collected summer in Ria Formosa were i) *C. edule*, *C. gigas* sampled in summer and *S. marginatus* collected in winter at the lagoon system; ii) *C. edule*, *R. decussatus* and *S. marginatus* sampled in summer at the Mondego estuary (Figure 4 and Appendix I).

S. plana, sampled at the Mondego estuary, showed similar protein content ($Z = 0.641$, $N = 12$, $p = 0.589$) in both seasons (2835.305 ± 99.638 µg/g in winter and 2576.883 ± 244.671 µg/g in summer). *S. plana* presented the third highest protein content in winter at the Mondego estuary. This species showed a protein content lower than *M. galloprovincialis* B in winter at the Mondego estuary, and higher than *R. decussatus* from both seasons and *C. edule* collected in summer at the estuarine system, *S. marginatus* sampled in winter and all species collected in summer at the Ria Formosa lagoon ($-2.242 < Z < 2.882$, $N = 12$, $0.002 < p < 0.026$). The species that did not demonstrate statistical differences with *S. plana* collected in winter at the Mondego estuary were i) *C. edule* sampled in winter, *M. galloprovincialis* B and *S. plana* collected in summer, *M. galloprovincialis* S and *S. marginatus* at both seasons at the estuarine system; ii) *C. edule*, *C. gigas*, *M. galloprovincialis* B and *R. decussatus* sampled in winter

at the Ria Formosa lagoon (Figure 4 and Appendix I). *S. plana* collected in summer at the Mondego estuary presented a protein content lower than *M. galloprovincialis* B sampled in winter at the Mondego estuary, and higher than *C. edule* collected in summer, at the estuarine system, and all bivalve species sampled in summer at the Ria Formosa lagoon ($-2.562 < Z < 2.882$, $N = 12$, $0.002 < p < 0.026$). The species that did not demonstrate statistical differences with *S. plana* collected in summer at the Mondego estuary were i) *C. edule*, *S. plana* and *S. marginatus* sampled in winter, *M. galloprovincialis* B collected in summer, *M. galloprovincialis* S, *R decussatus* and *S. marginatus* at both seasons at the estuarine system; ii) *C. edule*, *C. gigas*, *M. galloprovincialis* B, *R. decussatus* and *S. marginatus* sampled in winter at the Ria Formosa lagoon (Figure 4 and Appendix I).

S. marginatus showed higher protein content ($Z = 2.882$, $N = 12$, $p = 0.002$) in winter at the Ria Formosa lagoon than in summer (2123.963 ± 99.350 µg/g in winter and 884.561 ± 69.517 µg/g in summer). At the Mondego estuary, the protein content did not demonstrate significant differences ($Z = 1.121$, $N = 12$, $p = 0.310$), despite being higher in winter (2439.151 ± 208.880 µg/g in winter and 1724.489 ± 341.044 µg/g in summer). In summer, *S. marginatus* exhibited higher protein content at the Mondego estuary than at the Ria Formosa lagoon ($Z = 2.242$, $N = 12$, $p = 0.026$), while in winter, there was no significant difference detected ($Z = 1.121$, $N = 12$, $p = 0.310$), though in the Mondego estuary the protein content was higher than in the Ria Formosa lagoon. *S. marginatus* collected in winter at the Mondego estuary presented a protein content higher than *C. edule* sampled in summer at the estuarine system and all species sampled in summer at both study areas. Still, *S. marginatus* collected in winter at the Mondego estuary showed a lower protein content than *M. galloprovincialis* B sampled in winter at the estuarine system, *C. edule* and *C. gigas* sampled in winter at the lagoon system ($-2.2562 < Z < 2.882$, $N = 12$, $0.002 < p < 0.041$). The species that did not demonstrate statistical differences with *S. marginatus* collected in winter at the Mondego estuary were i) *C. edule* sampled in winter, *M. galloprovincialis* B and *S. marginatus* collected in summer, *M. galloprovincialis* S, *R. decussatus* e *S. plana* sampled in both seasons at the estuarine system; ii) *C. edule*, *M. galloprovincialis* B, *R. decussatus* and *S. marginatus* collected in winter at the lagoon system (Figure 4 and Appendix I). *S. marginatus* sampled in summer at the Mondego estuary presented lower protein content than *M. galloprovincialis* S and B and all species sampled in winter at the Mondego estuary and in Ria Formosa, respectively ($-2.722 < Z < -2.082$, $N = 12$, $0.004 < p < 0.041$). On the other hand, *S. marginatus* sampled in summer at the Mondego estuary showed higher protein content

than *M. galloprovincialis* B and *S. marginatus* sampled in summer in Ria Formosa ($Z = 2.242$, $N = 12$, $p = 0.029$). The species that did not demonstrate statistical differences with *S. marginatus* collected in summer at the Mondego estuary were i) *C. edule* and *R. decussatus* sampled in both seasons, *M. galloprovincialis* S and B and *S. plana* collected in summer at the estuarine system; ii) *C. edule, C. gigas* and *R. decussatus* sampled in summer and *S. marginatus* collected in winter at the Ria Formosa lagoon (Figure 4 Appendix I). *S. marginatus* collected in winter at the Ria Formosa lagoon presented i) lower protein content than *M. galloprovincialis* S and *S. plana* collected in winter and *M. galloprovincialis* B sampled in both seasons at the estuarine system, ii) lower protein content than all bivalve species collected in winter at the Ria Formosa lagoon, and on the other hand, iii) higher protein content than *C. edule* sampled in summer at the Mondego estuary and iv) higher protein content than *M. galloprovincialis* B, *R. decussatus* and *S. marginatus* collected in summer at the Ria Formosa lagoon ($-2.882 < Z < 2.882$, $N = 12$, $0.002 < p < 0.041$). The species that did not demonstrate statistical differences with *S. marginatus* collected in winter at Ria Formosa were i) *C. edule*, *C. gigas* and *R. decussatus* sampled in summer at the lagoon system, ii) *C. edule* and *S. plana* sampled in summer at the Mondego estuary, iii) *R. decussatus* and *S. marginatus* collected at both seasons at the estuarine system (Figure 4 and Appendix I). *S. marginatus* collected in summer at the Ria Formosa lagoon presented a protein content lower than i) all species sampled at both seasons at the Mondego estuary, ii) all bivalve species sampled in winter at Ria Formosa, iii) *C. edule* and *R. decussatus* sampled in summer at the lagoon system ($-2.882 < Z < -2.562$, $N = 12$, $0.002 < p < 0.009$). The species that did not demonstrate statistical differences with *S. marginatus* collected in summer at the Ria Formosa lagoon were *C. gigas*, *M. galloprovincialis* B and *R. decussatus* sampled in summer at the lagoon system (Figure 4 and Appendix I).

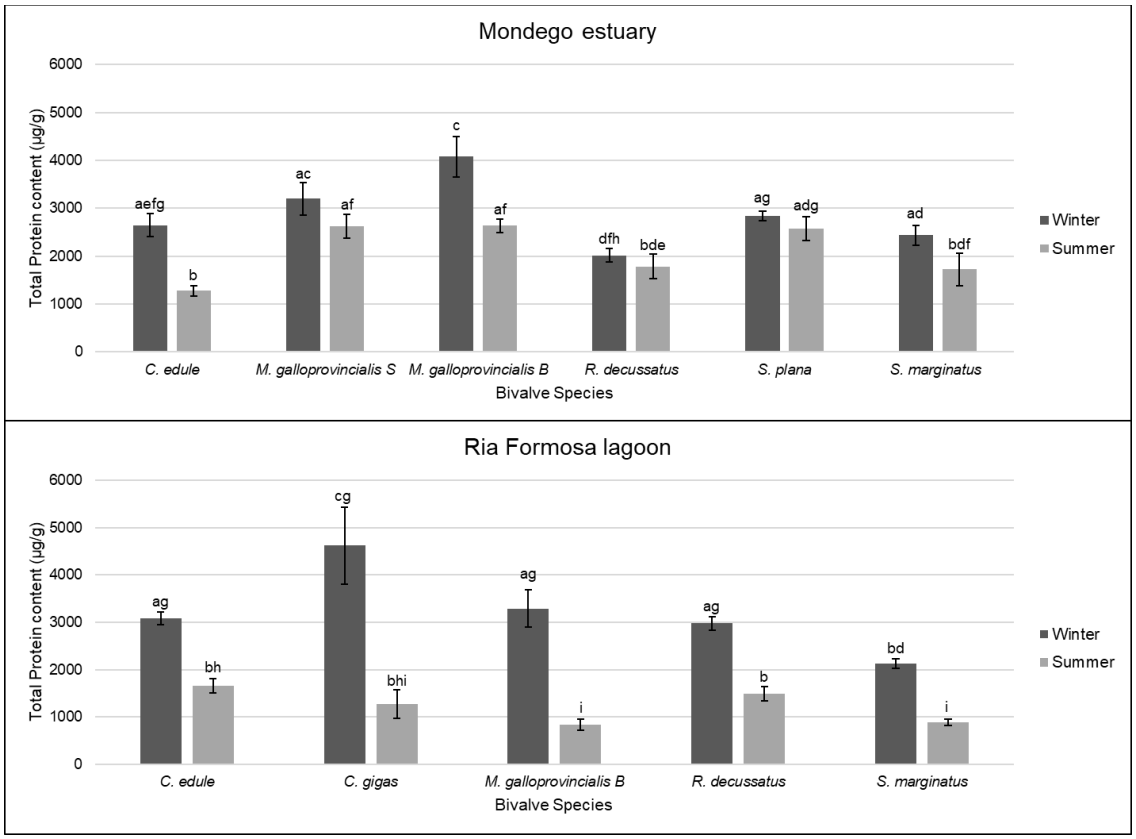

Figure 4. Total protein content of the bivalve species sampled at the Mondego estuary (A) and at the Ria Formosa lagoon (B), in winter 2016 (dark grey) and summer 2017 (light grey) seasons. Mean and standard error are shown in the data bars and error bars, respectively. The letters on the top of the bars stand for similar protein content ($p>0.05$). Different letters represent statistical differences between protein content ($p\leq0.05$) within each species, season and studied area.

3.3 Carbohydrate Composition

The bivalves' composition in polysaccharides residues was described for the species harvested in the Mondego estuary (Table 9) and in the Ria Formosa lagoon (Table 10), in winter and in summer. In general, all species from the Mondego estuary demonstrated to have higher polysaccharide content, particularly in winter, with the species *R. decussatus* showing the highest polysaccharide content, followed by the contents observed in *S. plana*, *M. galloprovincialis* S, *M. galloprovincialis* B and *C. edule*. While, in summer, *M. galloprovincialis* B and *R. decussatus* were the species with the highest polysaccharide concentrations (Table 9). The highest content in polysaccharides in the Ria Formosa lagoon was observed in *C. edule*, *R. decussatus* and *S. marginatus* in summer. In winter, *C. edule* was the species that showed the highest content, followed by *C. gigas* and *R. decussatus* (Table 10). In both studied areas and seasons, the most

abundant polysaccharide residue was glucose, that contributed, in average, to 79.51% of the total polysaccharide content. In much lower concentrations were detected xylose, that was the second main polysaccharide residue present in the bivalves, and other residues like rhamnose, fucose, ribose, arabinose, mannose, galactose and uronic acids (Tables 9 and 10).

Table 9. Abundance of polysaccharides residues (µg/g) of the bivalve species *C. edule*, *M. galloprovincialis* S (small size), *M. galloprovincialis* B (big size), *R. decussatus*, *S. plana* and *S. marginatus* harvested in the Mondego estuary in two different seasons (winter 2016 and summer 2017).

Species	*C. edule*		*M. galloprovincialis* S		*M. galloprovincialis* B		*R. decussatus*		*S. plana*		*S. marginatus*	
Season (µg/g) **Polysaccharides**	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Rhamnose	13.55	7.81		43.92		13.85	84.41	21.22	106.80	21.45		11.25
Fucose	39.10	13.57	741.02	119.67	125.05	21.28	218.27	163.10	119.32	4.25		11.17
Ribose	28.45	35.43	768.39	141.67	199.36	45.85	76.95	184.05	99.64	16.45	45.13	79.04
Arabinose	23.94	9.25		15.62	24.89	10.06	61.38	13.73	77.37	9.16	12.60	9.27
Xylose	179.26	10.84	337.14	144.93	105.74	121.22	266.33	162.73	299.12	35.32	186.89	27.48
Mannose	16.43	10.27	569.94	49.24	38.51	10.74	91.38	28.92	125.91	9.76	14.53	18.51
Galactose	24.93	20.15	927.32	90.74	104.50	20.78	154.54	205.38	149.59	6.37	28.38	23.71
Glucose	3718.70	349.17	3501.27	2012.65	5000.52	3192.29	9593.63	2656.73	7760.92	1087.60	3991.25	1455.55
Uronic Acids	14.68	35.76	690.21	157.65	109.63	82.79	60.20	37.26	55.34	190.14	44.59	10.04
Total	4059.04	492.25	7535.29	2776.09	5708.20	3518.86	10607.09	3473.12	8794.01	1380.50	4323.37	1646.02
N	9	9	7	9	8	9	9	9	9	9	7	9

Table 10. Abundance of polysaccharides residues (µg/g) of the bivalve species *C. edule*, *C. gigas*, *M. galloprovincialis* B (big size), *R. decussatus* and *S. marginatus* harvested in the Ria Formosa lagoon in two different seasons (winter 2016 and summer 2017).

Species	*C. edule*		*C. gigas*		*M. galloprovincialis* B		*R. decussatus*		*S. marginatus*	
Season (µg/g) **Polysaccharides**	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Rhamnose	7.19	43.35	2.06	2.97		4.23		19.33	1.23	8.29
Fucose	87.08	141.90	3.84	5.43	39.16	25.46	19.58	74.70	4.83	9.50
Ribose	115.84	287.16	8.55	12.63	24.42	39.02	14.21	146.20	19.24	61.49
Arabinose	14.76	50.72	0.94	4.75		3.63		12.630	2.15	6.58
Xylose	114.00	196.83	27.50	16.58	110.90		15.56	216.80	9.10	37.59
Mannose	41.86	75.39	2.94	23.85	7.58	10.96	11.98	51.02	7.34	17.33
Galactose	63.48	189.07	5.58	7.76	41.61	25.00	20.11	94.48	10.40	18.80
Glucose	1132.63	6481.07	752.87	571.96	218.19	176.06	687.20	2396.63	334.46	1424.75
Uronic Acids	85.65	31.93	29.01	9.15	13.95	20.11	29.17	14.87	19.63	7.36
Total	1662.49	7497.42	833.29	655.08	455.81	304.47	797.81	3026.66	408.38	1591.69
N	9	9	9	9	7	8	7	9	9	9

The two-dimensional n-MDS plot (Figure 5) shows an apparent distribution of the samples according to the studied areas, where they were harvested, and the different concentration and composition of the polysaccharide residues present in each sample (stress = 0.02). Three groups were defined. Group A included most of the species from the Mondego estuary that presented the highest abundance in polysaccharides. This group included *C. edule*, *M. galloprovincialis* B, *R. decussatus*, *S. plana* and *S. marginatus* collected in winter; *M. galloprovincialis* S, *M. galloprovincialis* B and *R. decussatus* sampled in summer, and some species from Ria Formosa, namely *C. edule* and *R. decussatus* collected in summer. *M. galloprovincialis* S sampled in winter, at the Mondego estuary, was not in this group because of the higher abundance in carbohydrates in contrast with the species included in it. Group B comprised the species that had a significant lower abundance of polysaccharides and it included *C. edule* collected in winter in the Ria Formosa lagoon, *S. plana* and *S. marginatus* sampled in summer at the Mondego estuary and at both studied sites, respectively. Group C comprised almost all species from the Ria Formosa lagoon that showed the lowest abundance in polysaccharides, which included *C. gigas*, *M. galloprovincialis* B, *R. decussatus* and *S. marginatus* collected in winter, *C. gigas* and *M. galloprovincialis* B sampled in summer, and a species from the Mondego estuary, collected in summer, *C. edule*.

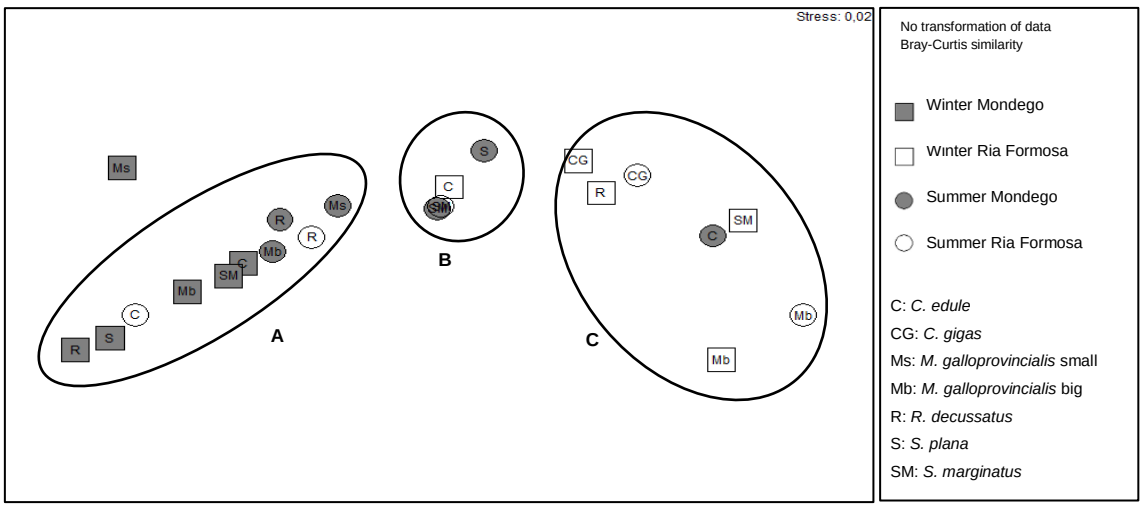

Figure 5. Two-dimensional non-metric multidimensional scaling (n-MDS) ordination plot of polysaccharide residues composition of the bivalve species sampled at the Mondego estuary and at the Ria Formosa lagoon, in winter (2016) and summer (2017) seasons. A, B and C are the groups defined in the n-MDS.

The ANOSIM analysis indicated a clear segregation of the three groups defined (R = 0.831; p = 0.001). The null hypothesis c) the polysaccharide residues profiles are equal between species and do not reveal spatial and seasonal variations was rejected. 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 = 0.710, p= 0.003; A/C: R = 0.994, p = 0.001; B/C: R = 0.664, p = 0.006). SIMPER analysis (Table 11) showed that in group A glucose followed by xylose explained 69.94% of the group similarity, in group B glucose followed by ribose explained 82.97% of the group similarity and in group C the polysaccharide residues glucose, uronic acids, ribose and galactose (in decreasing order of importance) explained 65.55% of the group similarity. Regarding the dissimilarities between groups, 51.57% of the dissimilarity among the groups A/B was explained by glucose, xylose and ribose, 78.29% of the dissimilarity between the groups A/C was explained by glucose and xylose and 51.12% of the dissimilarity among the groups B/C was explained by glucose, uronic acids, ribose and xylose. Glucose was the main polysaccharide residue that contributed both to the similarities within each group and the dissimilarities between the groups.

Table 11. Results of SIMPER analyses of abundance of polysaccharide residues showing average similarity and dissimilarity among the species inside each group according to non-metric multidimensional scaling (n-MDS) analysis.

Group	Average Similarity	Polysaccharide 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
Group	**Average Dissimilarity**	**Polysaccharide 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

C. edule (4059.04 µg/g and 492.05 µg/g of total polysaccharides in winter and in summer, respectively), *M. galloprovincialis* S (7535.29 µg/g and 2776.09 µg/g of total polysaccharides in winter and in summer), *S. plana* (8794.01 µg/g and 1380.50 µg/g of total polysaccharides in winter and in summer) and *S. marginatus* (4323.37 µg/g and 1646.02 µg/g of total polysaccharides in winter and in summer), collected at the Mondego estuary showed significant higher content in polysaccharides in winter, with the samples from both seasons, in general, being segregated at different n-MDS groups because of the dissimilarities between them. Despite having higher content in polysaccharides in winter, *M. galloprovincialis* B (5708.20 µg/g in winter and 3518.86 µg/g in summer) and *R. decussatus* (10607.09 µg/g in winter and 3473.12 µg/g in summer) collected at the Mondego estuary did not show significant differences among them, as the samples from both seasons were aggregated in the same n-MDS group. Also, in this studied area, small-size specimens of *M. galloprovincialis* showed higher concentration of total polysaccharides in winter and lower concentration in summer than the big-size organisms. In the Ria Formosa lagoon, *C. gigas* (833.29 µg/g in winter and 655.08 µg/g in summer) and *M. galloprovincialis* B (455.81 µg/g in winter and 304.47 µg/g in summer) demonstrated non-significant higher concentrations of polysaccharides in winter, with the samples from both seasons being aggregated in the same n-MDS group related with the similarities between them. On the other hand, *C. edule* (1662.49 µg/g in winter and 7497.42 µg/g in summer), *R. decussatus* (797.81 µg/g in winter and 3026.66 µg/g in summer) and *S. marginatus* (408.38 µg/g in winter and 1591.69 µg/g in summer) presented higher concentrations of polysaccharides in summer, being segregated in two distinct n-MDS groups. *C. edule* showed larger polysaccharide content at the Mondego estuary and at Ria Formosa lagoon, in winter and in summer, correspondingly. *M galloprovincialis* B, *R. decussatus* and *S. marginatus* presented higher polysaccharide content in the Mondego estuary than in the Ria Formosa lagoon, in both seasons.

The neutral sugar profile was described for the bivalve species harvested at the Mondego estuary (Table 12) and at the Ria Formosa lagoon (Table 13), in winter and summer. In general, all species from the Mondego estuary demonstrated to have higher neutral sugar abundance in summer, particularly *M. galloprovincialis* S and B and *R. decussatus* that presented the highest contents. While in winter, *M. galloprovincialis* S, *R. decussatus*, *M. galloprovincialis* B and *S. plana* were the species with the highest neutral sugar abundance (Table 12). In Ria Formosa, the highest content in neutral sugar was observed in *C. edule* and *R. decussatus* in summer, while in winter the highest content was observed in *C. edule* and *C. gigas* (Table 13). Considering the different

studied areas, *M galloprovincialis* S, *M. galloprovincialis* B and *R. decussatus* showed high neutral sugar concentrations at the Mondego estuary at both seasons. In Ria Formosa, at both seasons, *C. edule* showed higher neutral sugar abundance, followed by the neutral sugar contents of *C. gigas* in winter and *R. decussatus* in summer. In both studied areas and seasons, the most abundant neutral sugar was glucose, comprising, in average, 84.23% of the total neutral sugar content. Xylose was the second neutral sugar present in higher abundance in the bivalves' tissues collected at the Mondego estuary. Meanwhile, in Ria Formosa lagoon the second most abundant neutral sugar was fucose. The other neutral sugars detected were rhamnose, ribose, arabinose, mannose and galactose.

Table 12. Abundance of neutral sugars (µg/g) of the bivalve species *C. edule*, *M. galloprovincialis* S (small size), *M. galloprovincialis* B (big size), *R. decussatus*, *S. plana* and *S. marginatus* harvested in the Mondego estuary in two different seasons (winter 2016 and summer 2017).

Species	*C. edule*		*M. galloprovincialis* S		*M. galloprovincialis* B		*R. decussatus*		*S. plana*		*S. marginatus*	
Season (µg/g) **Neutral Sugars**	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Rhamnose	1.76	1.03	9.11	2.72	2.99	1.43		1.45				
Fucose	7.23	14.59	27.59	21.74	28.71	3.31	11.30	14.54	7.65	2.67		0.56
Ribose	1.09	21.25	47.60	26.52	12.78	5.15	7.09	1.27	12.27	11.20	0.88	5.97
Arabinose		28.51	8.19	28.96	0	26.67		33.16	6.32	13.77	0.36	0.76
Xylose	3.51		146.15	83.99	1.31	3.62	48.17	26.01	7.25	1.81		0.66
Mannose	0.47	1.43	6.30			2.75			1.65	2.63		1.10
Galactose	0.18	0.61	15.28		3.32	0.11	9.54	19.41	2.25	2.16		0.61
Glucose	94.34	258.52	208.46	755.21	145.82	726.87	354.77	411.05	154.48	172.26	91.67	134.24
Total	108.58	325.94	468.68	919.14	194.93	769.91	430.87	506.89	191.87	206.50	92.91	143.90
N	7	7	8	6	7	8	5	7	7	7	3	7

Table 13. Abundance of neutral sugars (µg/g) of the bivalve species *C. edule*, *C. gigas*, *M. galloprovincialis* B (big size), *R. decussatus* and *S. marginatus* harvested in the Ria Formosa lagoon in two different seasons (winter 2016 and summer 2017).

Species	*C. edule*		*C. gigas*		*M. galloprovincialis* B		*R. decussatus*		*S. marginatus*	
Season (µg/g) **Neutral Sugars**	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Rhamnose	39.60	1.55				0.50		0.67	1.17	0.70
Fucose	122.16	10.87			8.30	5.41		0.36	0.20	0.78
Ribose	48.93	36.75		0.98	2.14	9.80	50.11	2.12	2.37	2.12
Arabinose	25.41	27.41		0.27				8.57	0.21	3.56
Xylose	96.20	9.28	1.68	0.98			11.18	1.67	2.52	
Mannose	3.21	3.02		0.80			2.58		1.01	0.57
Galactose	16.75	4.44					1.05			
Glucose	1120.81	256.26	198.82	95.38	62.66	92.94	125.83	215.16	133.77	153.50
Total	1473.07	349.58	200.5	98.41	73.10	108.65	190.75	228.55	141.25	161.23
N	8	8	2	5	3	4	5	6	7	6

The two-dimensional n-MDS analysis (Figure 6) showed a separation of the samples based on the different concentration and composition of the neutral sugars present in each sample (stress = 0.03). Three groups were defined. Group A comprised the bivalve species that showed the lowest abundance of neutral sugars, including *C. edule* and *S. marginatus*, collected in winter at the Mondego estuary, and *C. gigas* and *M. galloprovincialis* B sampled in summer, in Ria Formosa. Group B was composed by species that presented a significant lower abundance of neutral sugars compared with species from group C, that included *M. galloprovincialis* B and *S. plana* collected in winter at the Mondego estuary, *S. plana* and *S. marginatus* sampled at the Mondego estuary in summer, *C. gigas*, *R. decussatus* and *S. marginatus* collected in winter at Ria Formosa, and *R. decussatus* and *S. marginatus* sampled in summer in Ria Formosa. Group C comprised the bivalve species that showed the highest abundance of neutral sugars, including *R. decussatus* collected in winter and *C. edule*, *M. galloprovincialis* (S and B) and *R. decussatus* sampled in summer, at the Mondego estuary, and also, *C. edule* collected in summer in Ria Formosa. *M. galloprovincialis* B sampled in winter at the Ria Formosa lagoon was not included in group A because of the lower abundance in neutral sugars in comparison with the species included in it. *M. galloprovincialis* S collected in winter, at the Mondego estuary, was not included in any group due to the distinct neutral sugar abundance that was higher than the abundance of the species present in groups A and B, and presenting a distinct composition compared to the species from group C. *C. edule* from Ria Formosa, sampled in winter, was not included in group C because of the higher abundance in neutral sugars in comparison with the species included in it.

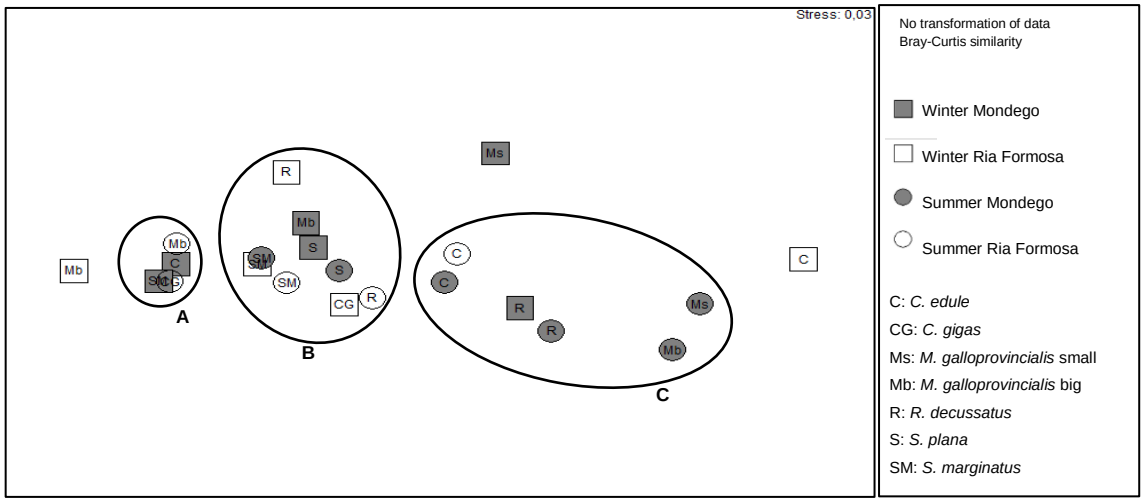

Figure 6. Two-dimensional non-metric multidimensional scaling (n-MDS) ordination plot of neutral sugar composition of the bivalve species sampled at the Mondego estuary and at the Ria Formosa lagoon, in winter 2016 and summer 2017. A, B and C were the groups defined in the n-MDS.

The ANOSIM analysis indicated a clear segregation of the three groups defined (R = 0.790; p = 0.001). The null hypothesis d) the neutral sugar profiles are equal between species and do not present spatial and seasonal variations was rejected. When comparing pairwise tests, all groups were significantly different ($p \leq 0.05$) and presented high R values, showing good segregation (A/B: R = 0.790, p= 0.001; A/C: R = 1, p = 0.005; B/C: R = 0.821, p = 0.002). SIMPER analysis (Table 14) showed that only glucose explained 92.83% of the similarity between samples in group A and 81.16% of the similarity within the group B. In group C glucose and arabinose (in decreasing order of importance) explained 69.15% of the group similarity. About the dissimilarities between groups, 31.10% of the dissimilarity among the groups A/B was explained by glucose, ribose, fucose and arabinose (in decreasing order of importance). 65.78% of the dissimilarity between the groups A/C was explained by glucose followed by arabinose and xylose; 48.59% of the dissimilarity among the groups B/C was explained by glucose, xylose, arabinose and ribose, in decreasing order of importance.

Table 14. Results of SIMPER analyses of neutral sugar abundance showing average similarity and dissimilarity among the species inside each group according to non-metric multidimensional scaling (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
Group	**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

C. edule (108.58 µg/g of neutral sugars in winter and 325.94 µg/g in summer), *M. galloprovincialis* S (468.68 µg/g of neutral sugars in winter and 919.14 µg/g in summer), *M. galloprovincialis* B (194.93 µg/g of neutral sugars in winter and 769.91 µg/g in summer) and *S. marginatus* (92.91 µg/g of neutral sugars in winter and 143.90 µg/g in

summer) from the Mondego estuary, showed significant higher neutral sugar content in summer, with the samples from both seasons being segregated in distinct n-MDS groups because of the dissimilarities between them. Despite having higher abundances in summer, *R. decussatus* (430.87 µg/g of neutral sugars in winter and 506.89 µg/g in summer) and *S. plana* (191.87 µg/g of neutral sugars in winter and 206.50 µg/g in summer), from the Mondego estuary, did not show significant differences between winter and summer, with the samples from both seasons being aggregated in the same n-MDS group because of the similarities between them. Also, at the Mondego estuary, small-size specimens of *M. galloprovincialis* showed higher neutral sugar concentrations in both seasons than big-size organisms. In the Ria Formosa lagoon, *C. edule* (1473.07 µg/g of neutral sugars in winter and 349.58 µg/g in summer) and *C. gigas* (200.50 µg/g of neutral sugars in winter and 98.41 µg/g in summer) demonstrated significant higher neutral sugar concentrations in winter, with the samples from both seasons being segregated in distinct n-MDS groups because of the dissimilarities between them, whereas *M. galloprovincialis* B (73.10 µg/g of neutral sugars in winter and 108.65 µg/g in summer) showed significant higher neutral sugar concentrations in summer. Despite presenting higher abundance of neutral sugars in summer, *R. decussatus* (190.75 µg/g in winter and 228.55 µg/g in summer) and *S. marginatus* (141.25 µg/g in winter and 161.23 µg/g in summer) from Ria Formosa did not show significant differences between winter and summer, with the samples from both seasons being aggregated in the same n-MDS group because of the similarities between them.

3.4 Fatty Acid Trophic Markers

Fatty acid trophic markers (FATMs) and diet preferences were determined to each bivalve species sampled at the Mondego estuary (Table 15) and at the Ria Formosa lagoon (Table 16), in the distinct sampling seasons (winter 2016 and summer 2017).

FATMs of *C. edule* indicated that this species is omnivorous at both studied sites and both seasons (Tables 15 and 16). DHA, C18:1n-9, C18:2n-6 and C20:1n-9, typical FATMs of a diet based on zooplankton, presented higher concentrations in winter and lower concentrations in summer. The DHA/EPA and C16:1n-7t/C16:0 ratios suggested that dinoflagellates were the preferable phytoplankton food source, instead of diatoms.

Nevertheless, diatoms and dinoflagellates contributed less to the diet of *C. edule*, than zooplankton. Furthermore, in *C. edule* specimens, the bacterial and detritus input contributed less to their diets than the zooplanktonic and phytoplanktonic input.

C. gigas, from the Ria Formosa lagoon, demonstrated an omnivorous behaviour in both seasons (Table 16). The high ratios of PUFAs/SFAs, and DHA/EPA and C16:1n-7t/C16:0 indicated a diet based on the consumption of phytoplankton, with a preference for dinoflagellates, zooplankton, bacteria and detritus. The last three food sources had a much lower contribution to their diet than the phytoplanktonic input. The decrease of the levels of DHA, EPA and other FATMs showed a presumably decrease of consumption of phytoplankton groups in summer.

Based on the FATMs of *M. galloprovincialis*, this species showed an omnivorous behaviour, with a diet based on zooplankton and phytoplankton with low input of bacteria and detritus, at both studied areas and seasons. In the Mondego estuary, small-size and big-size classes of organisms had higher concentrations of DHA, C18:1n-9, C18:2n-6 and C20:1n-9 in both seasons, which indicated a dominance of zooplankton and dinoflagellates on their diets, instead of diatoms (Table 15). In Ria Formosa, the big-size specimens showed a similar feeding behaviour in winter and summer (Table 16). However, the bacterial input in the lagoon system was lower in the winter and higher in the summer, unlike what occurred in the estuarine system.

R. decussatus had an omnivorous behaviour, since it presented high concentrations of phytoplankton and zooplankton FATMs at the Mondego estuary (Table 15). In winter, the diet was composed by zooplankton, dinoflagellates, the preferable phytoplankton choice, and diatoms. In the summer the pattern was the same, but the consumption of dinoflagellates decreased and the consumption of diatoms increased, still not surpassing the dinoflagellate preference. The bacterial input was lower than the phytoplankton and zooplankton input, but similar in both seasons. The consumption of detritus had a small contribution to these bivalves' dietary patterns. In the Ria Formosa lagoon, the concentration of the FATMs was lower but the preference for dinoflagellates and zooplankton, instead of diatoms, remained (Table 16). Also, the input of detritus was higher than the input of phytoplankton in both seasons, regarding the low PUFAs/SFAs ratio. Bacterial input had a small contribution to *R. decussatus* diet. The consumption of bacteria, dinoflagellates and detritus was higher in winter, while the consumption of diatoms was higher in summer.

Dietary preferences, reflected by the FATMs, indicated that *S. plana*, in the Mondego estuary, highlighted an omnivorous behaviour in winter and in summer (Table

15). The input of zooplankton was much higher than phytoplankton, in the winter season, like the DHA, C18:1n-9, C18:2n-6 and C20:1n-9 concentrations demonstrated. In summer, the consumption of zooplankton diminished, with the bivalves preferring dinoflagellates and diatoms. The consumption of detritus was higher in winter, but it was balanced with the consumption of phytoplankton (PUFA/SFA ratio approximately 1).

S. marginatus showed an omnivorous diet based on the consumption of zooplankton, phytoplankton, bacteria, and detritus in the winter. At the Mondego estuary the input of dinoflagellates and diatoms were balanced (Table 15), but in Ria Formosa there was a smaller input of diatoms (Table 16). In summer, at both studied areas, *S. marginatus* presented an herbivorous diet, which was indicated by the PUFA/SFA ratio and the low abundance of the zooplankton FATMs (C18:1n-9, C18:2n-6 and C20:1n-9). Dinoflagellates were the main food choice, indicated by DHA concentrations and low C16:1n-7t/C16:0 ratios. Bacteria and detritus contributed much less for the diet in summer than in winter.

Table 15. Fatty acid trophic markers (µg/g) of the bivalve species *C. edule*, *M. galloprovincialis* S (small size), *M. galloprovincialis* B (big size), *R. decussatus*, *S. plana* and *S. marginatus* sampled in the Mondego estuary, in winter 2016 and summer 2017.

Species	*C. edule*		*M. galloprovincialis* S		*M. galloprovincialis* B		*R. decussatus*		*S. plana*		*S. marginatus*	
Season / **FATMs** (µg/g)	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
PUFAs/SFAs	1.574	1.714	2.224	4.037	3.238	2.272	2.143	2.325	1.056	2.114	0.871	1.437
DHA	0.322	0.197	73.563	14.954	3.976	0.569	14.133	13.750	4.609	0.142	0.072	0.010
EPA	0.185	0.123	21.582	8.477	1.772	0.293	2.362	4.137	0.993	0.130	0.027	
DHA/EPA	1.743	1.608	3.409	1.764	2.243	1.938	5.984	3.324	4.643	1.097	2.653	
C16:1n-7/C16:0	1.031	1.132	0.069	0.815	0.065	0.196	0.254	0.249	0.284	0.578	2.039	0.716
C15:0+C17:0	0.102	0.050	3.404	0.624	0.205	0.085	0.667	0.698	0.701	0.029	0.066	0.007
C20:1n-9	0.020	0.013	24.018	5.627	0.483	0.078	0.565	0.667	0.520	0.057	0.005	
C18:1n-9	0.034	0.023	6.443	0.984	0.257	0.043	1.556	0.950	1.540	0.037	0.020	0.002
C18:2n-6	0.011	0.018	3.266	1.800	0.145	0.055	0.191	0.168	0.203	0.009	0.005	0.000

Table 16. Fatty acid trophic markers (µg/g) of the bivalve species *C. edule*, *C. gigas*, *M. galloprovincialis* B (big size), *R. decussatus* and *S. marginatus* sampled in the Ria Formosa lagoon, in winter 2016 and summer 2017.

Species	*C. edule*		*C. gigas*		*M. galloprovincialis* B		*R. decussatus*		*S. marginatus*	
Season / **FATMs** (µg/g)	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
PUFAs/SFAs	2.292	2.324	4.153	1.914	3.422	2.066	0.276	0.859	1.909	2.045
DHA	12.800	4.827	0.189	0.014	1.080	0.395	0.107	0.082	0.094	0.021
EPA	5.999	2.432	0.096	0.004	0.504	0.142	0.025	0.034	0.046	
DHA/EPA	2.134	1.984	1.970	3.801	2.144	2.788	4.244	2.432	2.070	
C16:1n-7/C16:0	0.133	0.450	0.420	0.816	0.251	0.624	0.388	0.674	0.389	0.464
C15:0+C17:0	0.773	0.330	0.011	0.007	0.079	0.122	0.332	0.076	0.037	0.007
C20:1n-9	0.633	0.322	0.019		0.187	0.039			0.007	0.002
C18:1n-9	1.006	0.455	0.031	0.005	0.089	0.028	0.011	0.012	0.011	0.001
C18:2n-6	0.408	0.079	0.016	0.002	0.033	0.024		0.021	0.008	0.006

The two-dimensional n-MDS plot (Figure 7) showed a distribution according to FATMs abundance and feeding preferences, despite not showing a clear distribution by species, site or season based on FATMs concentration and composition (stress = 0.04). However, three groups were defined. Group A comprised the species that presented an omnivorous behaviour with a higher tendency for phytoplankton consumption, namely dinoflagellates and diatoms, than for the consumption of zooplankton, that included *M. galloprovincialis* B*, R. decussatus* and *S. plana* collected in winter and *M. galloprovincialis* S and *R. decussatus* sampled in summer, all from the Mondego estuary, and *C. edule* collected at both seasons in the Ria Formosa lagoon. Group B comprised the species with omnivorous behaviour presenting a slightly higher tendency for phytoplankton consumption, mainly dinoflagellates, than for the consumption of zooplankton, that includes *C. edule* and *S. marginatus* collected in winter and *C. edule*, *M. galloprovincialis* B and *S. plana* sampled in summer, all from the Mondego estuary, and *C. gigas*, *M. galloprovincialis* B, *R. decussatus* and *S. marginatus* sampled in winter and *C. gigas*, *M. galloprovincialis* B and *R. decussatus* collected in summer in Ria Formosa lagoon. At group C were the *S. marginatus* specimens collected in summer at both studied sites that showed an herbivorous behaviour. *M. galloprovincialis* S sampled in winter at the Mondego estuary was not included in any group, despite presenting an omnivorous behaviour like most of the species studied, because it showed an homogeneous consumption of phytoplankton and zooplankton when comparing with the other omnivorous bivalve species in the groups A and B, respectively.

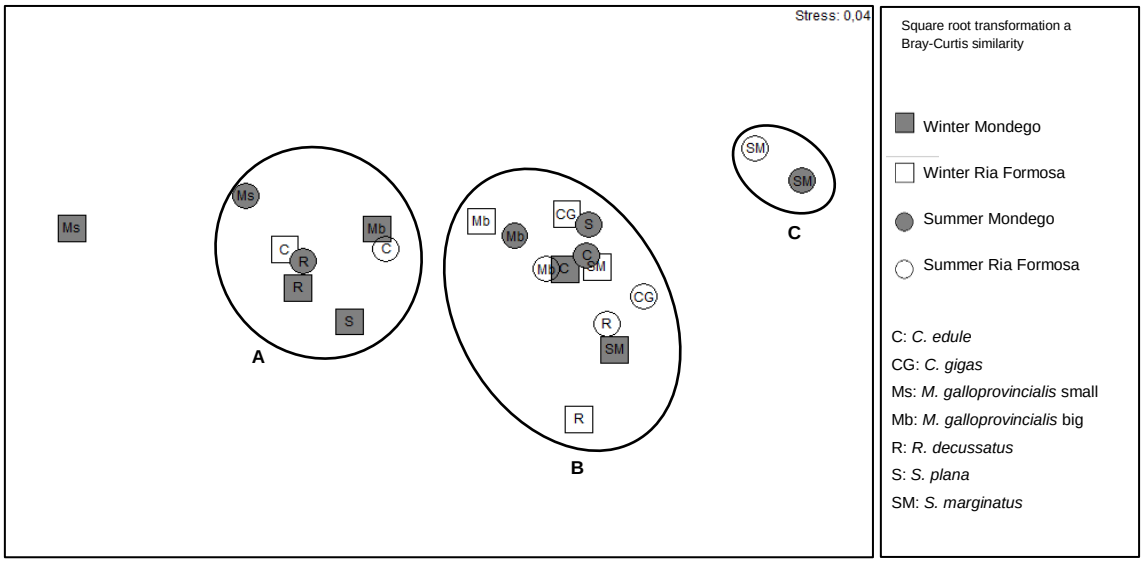

Figure 7. Two-dimensional non-metric multidimensional scaling (n-MDS) ordination plot of fatty acid trophic markers composition of the bivalve species sampled at the Mondego estuary and at the Ria Formosa lagoon, in winter and summer seasons. A, B and C were the groups defined in the n-MDS.

The ANOSIM analysis indicated a clear segregation of the three groups defined ($R = 0.961$; $p = 0.001$). The null hypothesis e) the bivalve species show the same food preferences was rejected. When comparing pairwise tests, all groups were significantly different ($p \leq 0.05$) and presented high R values, showing good segregation (A/B: $R = 0.952$, $p= 0.001$; A/C: $R = 1$, $p = 0.028$; B/C: $R = 0.963$, $p = 0.011$). SIMPER analysis showed that the trophic markers (in decreasing order of importance) that explained: i) 91.87% of the similarity in group A were DHA, DHA/EPA, EPA, PUFA/SFA, C18:1n-9, C20:1n-9, C15:0+C17:0; ii) 80.25% of the similarity in group B were DHA/EPA, PUFA/SFA, C16:1n-7t/C16:0, DHA, EPA, C15:0+C17:0; and iii) 88.48% of similarity within the group C were PUFA/SFA, C16:1n-7t/C16:0, DHA (Table 17). Regarding the dissimilarities between groups, the FATMs, in decreasing order of importance, that explained: i) 45.03% of the dissimilarity among the groups A/B were DHA, EPA, C18:1n-9, C20:1n-9, C15:0+C17:0, PUFA/SFA, C18:2n-6; ii). 69.92% of the dissimilarity between the groups A/C were DHA, EPA, DHA/EPA, C18:1n-9, C20:1n-9, C15:0+C17:0, C18:2n-6; and iii) 42.44% of the dissimilarity among the groups B/C were DHA/EPA, DHA, PUFA/SFA, EPA, C16:1n-7t/C16:0, C15:0+C17:0 (Table 18). DHA/EPA ratio, DHA and PUFA/SFA ratio were the FATMs that contributed most for the similarities within each group. DHA, DHA/EPA ratio and EPA were the main FATMs that contributed to the dissimilarities between the groups.

Table 17. Results of SIMPER analyses of fatty acid trophic markers abundance showing average similarity among the species inside each group according to non-metric multidimensional scaling (n-MDS) analysis.

Group	Average Similarity	Fatty Acids 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
		PUFA/SFA	2.49	11.72	5.21	14.27	70.12
		C18:1n-9	0.96	6.58	4.00	8.01	78.13
		C20:1n-9	1.26	5.82	8.71	7.08	85.21
		C15:0+C17:0	0.57	5.47	5.01	6.66	91.87
B	80.25	DHA/EPA	2.37	26.72	5.90	33.29	33.29
		PUFA/SFA	1.93	21.46	3.60	26.74	60.04
		C16:1n-7t/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
		C15:0+C17:0	0.08	3.70	2.46	4.61	93.55
C	88.48	PUFA/SFA	1.74	49.92	-	56.43	56.43
		C16:1n-7t/C16:0	0.59	28.37	-	32.07	88.50
		DHA	0.02	4.27	-	4.82	93.32

Table 18. Results of SIMPER analyses of fatty acid trophic markers showing average dissimilarity between sample groups according to non-metric multidimensional scaling (n-MDS) analysis.

Group	Average Dissimilarity	Fatty Acids 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
		PUFA/SFA	2.49	1.93	2.48	1.25	5.51	84.62
		C18:2n-6	0.43	0.02	2.48	1.68	5.51	90.13
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
		C15:0+C17:0	0.57	0.01	4.59	5.02	6.56	89.05
		C18:2n-6	0.43	0.00	3.41	2.14	4.88	93.92
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
		PUFA/SFA	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-7t/C16:0	0.71	0.59	2.85	1.20	6.71	84.64
		C15:0+C17:0	0.08	0.01	2.36	1.31	5.55	90.19

4. Discussion

The bivalve species studied in the present work revealed diverse biochemical composition, as it was expected for a seafood product (Larsen *et al*., 2011; Tacon *&* Metian, 2013). Seasonal changes in the biochemical composition were also highlighted at this study, corroborating the statements at previous studies of other bivalve species (Ansell, 1972; Ansell, 1974a, b; Walne *&* Mann, 1975; Zandee *et al*., 1980; Robert *et al*., 1993; Pérez Camacho *et al*., 2003). Most of the species from the Mondego estuary and the Ria Formosa lagoon showed a fatty acid content more diverse and with higher abundance in winter than in summer. In both studied areas and seasons, several fatty acids remained abundant among the bivalves FA profile. This was the case of the most abundant SFAs – palmitic acid (C16:0), heptadecanoic acid (C17:0) and octadecanoic acid (C18:0), – MUFAs – palmitoleic acid (C16:1 n-7 trans), 10-heptadecenoic acid (C17:1 n-8 cis), 11-eicosenoic acid (C20:1 n-9 cis), and 13-docosenoic acid (C22:1n-9 cis), – PUFAs – linoleic acid (C18:2 n-6 cis or LA) and α-linolenic acid (C18:3 n-3 cis or ALA), – and HUFAs – docosahexaenoic acid (C22:6 n-3 cis or DHA), eicosapentaenoic acid (C20:5 n-3 cis or EPA) and arachidonic acid (C20:4n-6 cis or ARA). PUFAs and HUFAs, were the main contributors to the FA profile of the studied bivalve species, followed by MUFAs and SFAs. The PUFAs and HUFAs that were found in greater concentrations in the bivalves were also considered to be essential fatty acids (EFAs), that are associated with several beneficial properties for the health of people that consume these molluscs and other seafood products with similar biochemical characteristics. The abundant fatty acids found in the edible bivalve species here studied were also described as the main contributors for FA composition in several bivalve species in previous studies (Karakoltsidis *et al*., 1995; Dridi *et al*., 2006; Prato *et al*., 2010; Ezgeta-Balić *et al*., 2012; Tacon *&* Metian, 2013; Gonçalves et al., 2016).

In terms of feeding preferences, almost all bivalve species, sampled in both studied areas and seasons, demonstrated a tendency for omnivory. Only *S. marginatus* showed an herbivorous behaviour in summer, at both estuarine and lagoon systems, and an omnivorous diet in winter. Ezgeta-Balić *et al*. (2012) stated that the fatty acid composition of the digestive glands of four bivalve species, including *M. galloprovincialis,* demonstrated a mixed diet, suggesting that the contribution of the different dietary components varied over the year in each species. The changes in dietary behaviour for phytoplankton, zooplankton, bacteria and detritus observed in the bivalve species in our

study 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.

In general, all bivalve species from the Mondego estuary and from the Ria Formosa lagoon demonstrated higher protein content in winter. Proteins are the major constituents of bivalves, assuming structural functions and as energy reserves, when glycogen levels are low, namely during gametogenesis (Matias *et al*., 2013).

The studied species from the Mondego estuary demonstrated to have higher polysaccharide content in winter, while in Ria Formosa the highest abundance was observed in summer, in *C. edule*, *R. decussatus* and *S. marginatus*, and in winter, in *C. gigas* and *M. galloprovincialis* B. In both studied areas and seasons, glucose was the most abundant polysaccharide residue. Glucose is stored in the bivalves' tissues in the form of glycogen, a polysaccharide with an important storage role in animals. Thus, the results of polysaccharide residues content indicated that glycogen was the main polysaccharide present in the bivalves tissues, which was is in accordance with previous researches (de Zwaan *&* Zandee, 1972; Pérez Camacho *et al.,* 2003; Matias *et al*., 2013). Other polysaccharide residue found in lower concentrations was fucose. Its presence could be explained by the existence of fucoidans in the bivalves' biochemical composition, provided by feeding in marine photosynthetic organisms. This polysaccharide was isolated from several marine invertebrate species, including sea cucumber (Ahmed *et al*., 2014).

In terms of neutral sugar profile, all species from the Mondego estuary demonstrated to have higher neutral sugar abundance in summer, while in the Ria Formosa lagoon only *M. galloprovincialis* B, *R. decussatus* and *S. marginatus* followed the same tendency. The neutral sugars, rhamnose, fucose, arabinose, xylose, mannose, and galactose, found in much lower abundance than glucose, are synthesized by marine algae, seaweeds and some microorganisms, but can be metabolized by bivalves and be a part of their biochemical composition (Ahmed *et al*., 2014; Kang *et al*., 2015). These neutral sugars could be free in the bivalves' cells, when they were sampled, as a result of them being transformed in several metabolic pathways to form polysaccharides.

Seasonal variability of nutrient abundance in the bivalve species could result from several environmental factors, like temperature, food availability, food composition and pollutants, as well as from physiological factors, like mobilization of nutrients, energy storage and utilization during the reproductive cycle. Biochemical composition also

varies according to geographical location and species (Sastry *et al*., 1979; Navarro *et al*., 1989; Gosling, 2003; Gonçalves *et al*., 2016)

The reproductive cycles of *C. edule* suggested by Navarro *et al*. (1989) and Newell *&* Bayne (1980) in the Mundaca estuary (Spain) and in the Tamar estuary (England), respectively, were composed by gamete formation starting in winter until early spring, when the improvement of food availability in the environment is able to supply sufficient energy to endure gametogenesis. Until spawning, these organisms suffer severe metabolic costs. After spawning in summer, the metabolic and feeding rates decline. But, as food availability is still high after this period, the specimens continue to feed and can accumulate new energy reserves, specially composed by carbohydrates, to be mobilized for another gametogenesis process when the resting stage is finished. These authors proposed that environmental changes could influence the occurrence of reproductive stages in a single population, meaning that these stages could occur in distinct periods over the years. At the present study, the fatty acid profile of *C. edule*, sampled in the Mondego estuary, was more diverse than the ones described in specimens from the same studied area by Gonçalves *et al*. (2016) and Mesquita *et al*. (2018). The samples of *C. edule* showed spatial variations between the FA profile at the Mondego estuary and in the Ria Formosa lagoon, with significant higher concentrations in the last studied area, in both seasons, probably because of better environmental conditions and food availability. At the Mondego estuary, the high FA abundance in winter was not considered significant different from the abundance in summer. This species also established a seasonal variation in Ria Formosa, with higher FA concentrations in winter, that coincide with the period of resting stage or beginning of gamete formation, highlighting the good energetic reserves and the best nutritious condition of the specimens. Based on FATMs, *C. edule* showed an omnivorous behaviour, with the consumption of phytoplankton and zooplankton, which is in accordance to the findings of Mesquita *et al*. (2018). *C. edule* was the bivalve species with the greatest FA content in both studied seasons in Ria Formosa lagoon. In terms of total protein content, *C. edule* specimens showed a seasonal variation, with significantly higher protein content in winter than in summer, at both studied areas, as expected. In winter, the protein reserves are higher due to the accumulation of nutrients provided by the increase of food consumption after spawning and during the resting stage. Still, *C. edule* was the bivalve species that showed the highest protein content in summer, in Ria Formosa. On the other hand, *C. edule* did not present a spatial variation of protein content among the studied areas. Considering polysaccharide content, a seasonal

variation was observed to *C. edule* at the Mondego estuary and at the Ria Formosa lagoon, with higher abundance in winter and in summer, respectively. Still, comparing to the studied bivalve species, *C. edule* presented the highest content of polysaccharides in the Ria Formosa lagoon, in both seasons. Furthermore, *C. edule* showed a seasonal variation of neutral sugars at the Mondego estuary, presenting higher abundance in summer, that may be related with the need to store polysaccharide reserves to be used in gamete formation; in Ria Formosa, the species presented higher abundance in winter probably related with the metabolic synthesis of carbohydrates acquired and stored from diet. In terms of neutral sugar content, a spatial variation was observed in winter, with *C. edule* presenting higher abundance in the Ria Formosa lagoon. Indeed, *C. edule* was the bivalve species with the greatest neutral sugar content in both seasons, in the Ria Formosa lagoon.

C. gigas was collected only in the Ria Formosa lagoon presenting seasonal variation of FA content, showing higher FA content and diversity in winter than in summer. These results were in accordance with the work of Dridi *et al*. (2007), that stated seasonal variations at biochemical composition of gonad-visceral mass of *C. gigas* from the Bizert lagoon, Tunisia, and described an accumulation of fatty acids in gonads during the period of maturation of the species, in spring, followed by a decrease in summer and an increase in autumn, as a result of a rise in food availability. However, Pogoda *et al*. (2013) observed an opposite trend, an increase on fatty acid abundance in offshore-cultivated oysters in summer. Based on FATMs, the FA composition of *C. gigas* suggests an omnivorous diet, with a preference for dinoflagellates and zooplankton. Although, a study at the Akkeshi-ko estuary, in Japan, verified that diatoms were the most abundant phytoplankton found in the gut of *C. gigas* (Kasim *&* Mukai, 2009). Considering the protein content, a seasonal variation was observed to *C. gigas* that presented a significantly higher content in winter than in summer, revealing to be the bivalve species with the highest protein content in winter in the Ria Formosa lagoon. Comparing to the results of Dridi *et al*. (2007), these authors observed high protein levels in the gonad-visceral mass of the organisms in May and July, which corresponded to gamete maturation. The specimens studied by Pogoda *et al*. (2013) demonstrated an increase of total protein in spring and a decrease in late summer, whereas, in the present study, *C. gigas* presented a lower protein content in summer, which could have been mobilized to the gonads to endure gamete's formation and maturation. Dridi *et al.* (2007) also observed low levels of protein content in late summer, which corresponded to the beginning of spawning. These protein levels may increase in autumn, after the spawning

period is finished, and the bivalves enter the resting stage, when the food consumption rate increases as the food is still available in the ecosystem (Dridi *et al.,* 2007). *C. gigas* did not demonstrate any significant seasonal variation in polysaccharide content, despite the higher abundance in winter, but showed a significant seasonal variation of neutral sugar abundance, presenting a higher abundance in winter. Indeed, *C. gigas* presented the second highest polysaccharide content in Ria Formosa, in winter, compared to the studied bivalve species, and glycogen, composed by residues of glucose, was the main polysaccharide found in this species. Dridi *et al*. (2007) demonstrated that, in general, the biomolecules' abundance in the gonads decreased in summer months, which was corroborated by the present work based on the biochemical quantifications in the body tissues of the studied specimens used. Furthermore, the maximum concentrations of glycogen observed in winter emphasised the support given to gametogenesis, whereas the abruptly decrease to low values during summer may be explained by the spawning events occurrence. In contrast to what was observed in the present work, Pogoda *et al.* (2013) reported an increase in glycogen, in the offshore-cultivated oysters, from spring to early summer, which was related to high food abundance in these seasons, when phytoplankton blooms may occur.

Small-size (S) class of *M. galloprovincialis* from the Mondego estuary showed seasonal variation of FA profiles, presenting higher FA content in winter than in summer. Big-size (B) organisms of *M. galloprovincialis* showed a significant seasonal variation only at the Mondego estuary, with higher abundance in FA concentrations in winter. Contrarily, Prato *et al*. (2010) observed higher fatty acid content in summer and higher contribution of SFAs than of PUFAs and HUFAs, while Martínez-Pita *et al.* (2012) observed an increase on fatty acid abundance, specially n-3 PUFAs, during gonad maturation, which generally starts in winter and prolongs until summer. *M. galloprovincialis* B demonstrated a spatial variation in winter, presenting a higher FA composition at samples from the Mondego estuary than from the Ria Formosa lagoon. Comparing the different sizes of *M. galloprovincialis* from the Mondego estuary, the smaller organisms presented higher FA content than the bigger organisms. Based on FATMs composition, *M. galloprovincialis* demonstrated an omnivorous diet, with a low input of bacteria and detritus, to both studied areas and seasons. This was in accordance with the work of Prato *et al*. (2010) that studied the same species. *M. galloprovincialis* was the bivalve species with the greatest FA profile observed at the Mondego estuary and with the second greatest profile at the Ria Formosa lagoon. In terms of total protein content, *M. galloprovincialis* S from the Mondego estuary did not show a significant

variation between winter and summer, whereas *M. galloprovincialis* B from both studied areas showed seasonal variation of protein content, presenting higher content in winter, which could be explained by an increase of food consumption after the high availability of food in autumn (Martínez-Pita *et al*., 2012). Within each season, small-size and big-size individuals of *M. galloprovincialis* from the Mondego estuary did not have a significant variation between each size, despite big-size organisms presenting higher protein content than small-size bivalves to both seasons. Only in summer *M. galloprovincialis* B showed a spatial variation in protein content, with the bivalves from the Mondego estuary presenting higher protein content. Both sizes of *M. galloprovincialis* collected at the Mondego estuary showed the highest protein content in winter and summer. Concerning the polysaccharide profile, *M. galloprovincialis* S showed a significant seasonal variation, with higher content in winter. This could be explained by an increase in the storage of glucose as glycogen in the sexual resting stage, as the rise of food consumption occurs so that mussels are able to initiate gametogenesis in winter (Martínez-Pita *et al*., 2012). The lower polysaccharide abundances may correspond to a depletion after gametogenic formation and ripeness, taking place in spring and summer (Martínez-Pita *et al*., 2012). As for *M. galloprovincialis* B, it did not reveal a seasonal variation of polysaccharide residues in any of the studied areas. On the other hand, it demonstrated spatial variation of polysaccharide residues in winter and summer, with higher abundances at the Mondego estuary. In both size classes of *M. galloprovincialis* sampled at the Mondego estuary was observed a high polysaccharide content. In fact, this species presented the second highest abundance in carbohydrates, in winter, while in summer, the big-size specimens had the highest abundance of all species and the small-size specimens had the third highest abundance, to the reported studied area. In terms of neutral sugar abundance, *M. galloprovincialis* S showed a seasonal variation, presenting higher abundance of neutral sugars in summer. *M. galloprovincialis* B from both studied areas demonstrated the same seasonal variation, with higher abundance in summer. As for spatial variation of neutral sugar profile, it was observed a significant higher abundance in samples from the Mondego estuary, harvested in both seasons. According to a previous study that used this species (Martínez-Pita *et al*.,2012), the stored energy starts to be depleted during gametogenesis, gonadal development and maturation, with glycogen being broken down into glucose. Glucose and other neutral sugars are free in the mussels' body as they are mobilized to gametes to form their own energetic reserves. In these stages, it is expected to find higher content of glucose and other neutral sugars free in the organism. Both sizes of *M. galloprovincialis,* collected at

the Mondego estuary demonstrated to have the greatest neutral sugar content in summer and small-size specimens, from the same studied area, showed the highest neutral sugar content in winter.

R. decussatus showed a spatial variation of FA content in both seasons, showing a higher FA composition at the Mondego estuary than in the Ria Formosa lagoon. In fact, the second bivalve species with the greatest FA composition from the estuarine system was *R. decussatus*. This species also showed a seasonal variation in Ria Formosa, with higher FA concentrations in winter season. A reason for the high FA abundance of *R. decussatus* at the Mondego estuary, in winter and summer, could be due to a low density found when the sampling campaigns occurred and, consequently, the low number of replicates sampled that led to a division of the organisms in equal parts (sub-replicates) so that all biochemical analysis, including FA analysis, could be accomplished. A higher representative sampling of this species may show some FA variance or corroborate the high FA content observed. Based on FATMs, the FA composition of *R. decussatus* suggested an omnivorous diet, with the input of zooplankton, phytoplankton and detritus. In Ria Formosa, *R. decussatus* did not show a diverse PUFA content in summer, and especially in winter, which could explain why the input of detritus surpassed phytoplankton in this season. As reported in literature, the reproductive cycle of this species is composed by a gametogenesis and ripe stage in spring, followed by spawning that starts in late spring and ends in early autumn, and a resting stage from autumn to winter (Ojea *et al*., 2004; Matias *et al*., 2013). The reproductive stages, along with environmental stressors like water temperature, salinity and food availability, have an impact on the biochemical composition of *R. decussatus* along the year, namely carbohydrates that have an important role in gamete formation and survival of adult organisms during periods of nutritive stress in the reproductive cycle (Camacho *et al*., 2003; Matias *et al*., 2013; Aru *et al*., 2017). Considering the total protein content, *R. decussatus* showed a clear seasonal variation in the Ria Formosa lagoon, presenting a higher protein amount in winter, while the specimens that were in resting stage, recovering from the reproductive stress, endured during summer. In the Mondego estuary it was not observed any seasonal variation. A spatial variation of total protein content was detected only in winter, with the highest values registered in the Ria Formosa lagoon. In terms of polysaccharide content, *R. decussatus* showed a seasonal variation only in Ria Formosa, reporting a significant higher abundance in summer, which was not expected, since in this period the nutritive stress is higher due to the energetic investments in the reproductive success. It was demonstrated a spatial variation in

winter, with higher abundance of polysaccharide residue in the Mondego estuary, as expected for organisms that were currently in resting stage as reported in a previous study (Aru *et al*., 2017). *R. decussatus* was the species that showed the highest content of polysaccharide residues in winter, at the Mondego estuary. Whereas in the lagoon system, it presented the second highest polysaccharide residues content, in summer. Considering the content in neutral sugars, *R. decussatus* did not show significant changes among seasons, to both studied areas, despite presenting slightly higher content in summer. This was probably due to the neutral sugars that were being mobilized, in winter, to the starting of gamete formation and, in summer, to overcome the stress induced by energetic depletion during spawning. Looking at each season, *R. decussatus* showed a significant spatial variation as the abundance of neutral sugars was higher in the Ria Formosa lagoon than in the estuarine system. This species showed the most abundant neutral sugar composition in winter at the Mondego estuary, and it was the second species with greatest abundance in summer. *R. decussatus* was also the species from Ria Formosa with highest neutral sugar abundance in both seasons. Aru *et al*. (2017) reported that the biochemical composition could vary differently during the reproductive cycle, according to the sex of the organism, which is an important variable to consider in similar future studies.

The reproductive cycle of *S. plana* was studied in Guadalquivir estuary, in Spain, and it comprised a gametogenesis and ripe stage from late winter to late summer, a spawning stage in spring and in summer, when the water temperature was higher, and a resting stage from autumn until winter, when the water temperature was lower (Rodriguéz-Rúa *et al*., 2003). *S. plana*, sampled only in the Mondego estuary, showed a seasonal variation of the FA profiles, presenting a significant higher content in winter than in summer, as expected in a resting stage, when the specimens are resetting the biochemical composition and the energy stored after being depleted during gamete formation, maturation and spawning periods. The diversity of fatty acids was similar to other specimens studied in the same estuarine area of the present work (Gonçalves *et al.*, 2016), and higher than the ones described in the Blavet and Goyen estuaries and in the Bay of St. Brieuc (France) by Perrat *et al.* (2013). Based on FATMs, the FA composition of *S. plana* indicated an omnivorous diet, consuming zooplankton, phytoplankton and a small portion of detritus, being in agreement with Gonçalves *et al.* (2016). The same authors described that specimens of *S. plana* collected in the Mondego estuary were more nutritive than *C. edule*, with the richest FA profile, which was contradictory with the findings of this work. This could be explained by the

environmental conditions and stressors that these species are subject and that are capable of influencing the biochemical pathways, which highlights the dynamics of the estuarine system (Gonçalves *et al*., 2012). Considering total protein content, *S. plana* did not show a significant seasonal variation between winter and summer. Even though the content observed in winter was slightly higher than the content reported in summer, which suggested that protein stored in these specimens was not mobilized to gamete formation and maturation, in summer, while glycogen reserves were used in these stages, as showed by the significant seasonal variation of polysaccharides content, showing lower abundance in summer. The neutral sugar content presented similar profiles in both seasons, which could indicate that in winter they were obtained from food and were stored as polysaccharides, whereas in summer they were mobilized from these stored polysaccharides to gamete and gonadal maturation (Rodriguéz-Rúa *et al*., 2003).

Despite, in literature, there are some studies about the biochemical composition of *S. marginatus* during the larval development (da Costa *et al*., 2011; Aranda-Burgos *et al*., 2014), information about changes in biochemical composition of adult specimens is scarce. According to the reproductive cycle studied in Spain (Remacha-Triviño *&* Anadón, 2006) and Tunisia (Ayache *et al*., 2016), the resting stage starts in summer until autumn, gonad maturation starts in late summer until early winter, gametogenesis occurs in winter and spring, ripe stage occurs during spring, and spawning occurs in summer. Depending on the geographical location, these stages may occur earlier or later according to what the previous studies described. In the present work, *S. marginatus* did not show a spatial variation of FA content, in summer, between the Mondego estuary and the Ria Formosa lagoon, but showed a seasonal variation on the FA at the estuarine system, presenting higher content in winter. Similarly to what happened with the other species studied, the seasonal variation of fatty acid abundance could be explained by an increase of food consumption, while recovering from the reproductive stress in summer. Based on FATMs, the FA composition of *S. marginatus* indicated an omnivorous behaviour in winter and a preference for phytoplankton (herbivory diet) in summer. *S. marginatus* showed a significant seasonal variation in protein content, in the Ria Formosa lagoon, with higher content in winter. At the Mondego estuary, the total protein content, in winter, was considered non-significantly higher than the total protein content in summer. This could indicate that the reserves of protein at the estuarine system were partially used in summer, when this species was in ripeness and spawning stages, explaining the seasonal variation in this area. In the Ria Formosa lagoon, the specimens did not show significant seasonal variation in protein content but presented a higher

amount in carbohydrates reserves in summer, despite the spawning stage and, consequently, the high stress endured, probably due to a high phytoplankton availability that could have occurred during spring, and a reduction of zooplanktonic organisms, explaining the change of the feeding behaviour in this area. In summer, this species exhibited a spatial variation, with higher protein content in the Mondego estuary than in the lagoon system. In winter, it did not show a significant spatial variation, despite the higher content in the Mondego estuary rather than in the Ria Formosa lagoon. In terms of polysaccharide residues, *S. marginatus* demonstrated a seasonal variation in the Mondego estuary, with higher abundance in winter and, in Ria Formosa, with higher abundance in summer. It also showed a spatial variation in winter, with higher abundance of polysaccharide residues at the Mondego estuary than in Ria Formosa, probably due to a resting stage with greater food abundance at the estuarine system than at the lagoon. *S. marginatus* showed a seasonal variation in the neutral sugar content only at the Mondego estuary, with significant higher abundance in summer, which may be explained by the depletion of these components from the carbohydrate reserves to diminish the stress induced during spawning stage. *S. marginatus* also showed significantly higher abundance in neutral sugar content in winter, in Ria Formosa, most likely related with the stored energy, coming from carbohydrates obtained by food consumption during the resting stage, being mobilized to gamete formation (Remacha-Triviño *&* Anadón, 2006; Ayache *et al*., 2016).

Considering all components of the biochemical composition (fatty acids, total protein, polysaccharide residues and neutral sugars) assessed in this work, *M. galloprovincialis* and *R. decussatus* appeared to have better nutritious composition, presenting higher fatty acids, especially essential fatty acids, total protein, polysaccharide residues and neutral sugar contents at the Mondego estuary. On the other hand, in the Ria Formosa lagoon, *C. edule*, *C. gigas* and *R. decussatus* revealed a better nutritious composition, with higher fatty acid, total protein, polysaccharide residues and neutral sugar contents. The species mentioned are pointed out as being the best choices for a healthy human diet and being confirmed as a reliable choice for harvesting and production in aquacultures.

5. Conclusions

The present study highlights that, in general, bivalves freshly harvested in summer show less nutritive value, possibly as a result of reproductive effort (ripeness and spawning). It is here suggested the consumption of these bivalves in winter months, when these organisms have already recovered from the reproductive effort and there is an increase of food availability in water, after highly productive warmer season. However, it is important to bear in mind that these molluscs can accumulate toxic microorganisms and compounds (Aru *et al*, 2018), thereby the consumption must be carefully conducted and always evaluate the potential pollutants accumulated by the bivalve edible species. Furthermore, the consumers must know the provenance of the seafood products and if they were harvested in periods of no interdictions (another issue that must be carefully inspected by authorities).

At the Mondego estuary, the species *M. galloprovincialis* and *R. decussatus* showed the highest nutritional value, mainly in winter. In the Ria Formosa lagoon, *C. edule* and *R. decussatus* presented the highest nutritive content, in both seasons, with great fatty acid, protein, polysaccharide residues and neutral sugar contents. Also, *C. gigas* showed higher nutritive value in summer, with greater protein and polysaccharide residues contents.

The results obtained in this study will serve as additional information to the literature about nutritive composition of commercial important bivalve species. These results may be of interest to local farmers, stakeholders in aquaculture or other areas of production and management, as this knowledge could be applied in the selection, production and maintenance of commercial bivalve species in order to optimize their business by increasing the food quality. Furthermore, it can be used to add economic value to the species demonstrated in this study to have superior nutritional quality, that are already being commercially explored in the geographical studied locations, which is a financial benefit for producers and stakeholders. These results may be also of interest to consumers, so that they can understand the real nutritive value of the most consumed bivalve species, to be able to choose i) the products that grant better health benefits, ii) the best season to consume them, as well as iii) the area that produces bivalves with better nutritive quality.

Moreover, at future research studies, sampling campaigns to collect bivalve organisms and potential food sources should be conducted in each season, for a longer

period than a year, to better understand the seasonal variations and correlations of biochemical content among organisms at distinct levels of the trophic food web, like preys and predators or producers and consumers. A deeper knowledge in the intake process and at the metabolic synthesis of specific biomolecules, mainly the so called essential nutrients, together with the explanation of the mechanistic actions in the life cycle of edible bivalve species, will allow to get a more precise and detailed information about the ecological role of these species at the trophic food web and to help the consumers to identify important nutritious benefits for their health.

6. Appendixes

Appendix I. Results of the pairwise two-tailed Mann-Whitney U tests (U is the Mann-Whitney U-value, Z is the Z-value and p is the p-value) performed between the bivalve species sampled in both seasons, winter 2016 and summer 2017, and at both study areas, the Mondego estuary and the Ria Formosa lagoon, to estimate which groups have significant different ($p \leq 0.05$) distributions of total protein content (grey cells). Total protein content (mean ± standard error µg/g) of each sample is shown in the last row of the table. S* means small organisms; B** means big organisms.

Study Area			**Mondego estuary**											
	Species		*C. edule*		*M. galloprovincialis* S*		*M. galloprovincialis* B**		*R. decussatus*		*S. plana*		*S. marginatus*	
		Season	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Mondego estuary	*C. edule*	Winter		U = 1 Z = -2.72 p = 0.00	U = 12 Z = 0.96 p = 0.39	U = 15 Z = -0.48 p = 0.70	U = 2 Z = 2.56 p = 0.01	U = 18 Z = 0.00 p = 1.00	U = 5 Z = -2.08 p =0.04	U = 6 Z = -1.92 p = 0.06	U = 15 Z = 0.48 p = 0.70	U = 17 Z = -0.16 p = 0.94	U = 12 Z = -0.96 p = 0.39	U = 6 Z = -1.92 p = 0.06
		Summer	U = 1 Z = 2.72 p = 0.00		U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 1 Z = 2.72 p = 0.00	U = 9 Z = 1.44 p = 0.18	U = 0 Z = 2.88 p = 0.00	U = 1 Z = 2.72 p = 0.00	U = 1 Z = 2.72 p = 0.00	U = 15 Z = 0.48 p = 0.70
	M. galloprovincialis S*	Winter	U = 12 Z = -0.96 p = 0.39	U = 0 Z = -2.88 p = 0.00		U = 11 Z = -1.12 p = 0.31	U = 11 Z = 1.12 p = 0.31	U = 13 Z = -0.80 p = 0.48	U = 2 Z = -2.56 p = 0.01	U = 3 Z = -2.40 p = 0.12	U = 16 Z = -0.32 p = 0.82	U = 11 Z = -1.12 p = 0.31	U = 10 Z = -1.28 p = 0.24	U = 5 Z = -2.08 p = 0.04
		Summer	U = 15 Z = 0.48 p = 0.70	U = 0 Z = -2.88 p = 0.00	U = 11 Z = 1.12 p = 0.31		U = 3 Z = 2.40 p = 0.02	U = 14 Z = 0.64 p = 0.59	U = 6 Z = -1.92 p = 0.06	U = 5 Z = -2.08 p = 0.04	U = 9 Z = 1.44 p = 0.18	U = 16 Z = -0.32 p = 0.82	U = 17 Z = -0.16 p = 0.94	U = 8 Z = -1.60 p = 0.13
	M. galloprovincialis B**	Winter	U = 2 Z = -2.56 p = 0.01	U = 0 Z = -2.88 p = 0.00	U = 11 Z = -1.12 p = 0.31	U = 3 Z = -2.40 p = 0.02		U = 3 Z = -2.40 p = 0.02	U = 0 Z = -2.88 p = 0.00	U = 1 Z = -2.72 p = 0.00	U = 4 Z = -2.24 p = 0.03	U = 2 Z = -2.56 p = 0.01	U = 2 Z = -2.56 p = 0.01	U = 1 Z = -2.72 p = 0.00
		Summer	U = 18 Z = 0.00 p = 1.00	U = 0 Z = -2.88 p = 0.00	U = 13 Z = 0.80 p = 0.48	U = 14 Z = -0.64 p = 0.59	U = 3 Z = 2.40 p = 0.02		U = 4 Z = -2.24 p = 0.03	U = 5 Z = -2.08 p = 0.04	U = 11 Z = 1.12 p = 0.31	U = 16 Z = -0.32 p = 0.82	U = 15 Z = -0.48 p = 0.70	U = 8 Z = -1.60 p = 0.13
	R. decussatus	Winter	U = 5 Z = 2.08 p =0.04	U = 1 Z = -2.72 p = 0.00	U = 2 Z = 2.56 p = 0.01	U = 6 Z = 1.92 p = 0.06	U = 0 Z = 2.88 p = 0.00	U = 4 Z = 2.24 p = 0.03		U = 11 Z = -1.12 p = 0.31	U = 0 Z = 2.88 p = 0.00	U = 9 Z = 1.44 p = 0.18	U = 9 Z = 1.44 p = 0.18	U = 13 Z = -0.80 p = 0.48
		Summer	U = 6 Z = 1.92 p = 0.06	U = 9 Z = -1.44 p = 0.18	U = 3 Z = 2.40 p = 0.12	U = 5 Z = 2.08 p = 0.04	U = 1 Z = 2.72 p = 0.00	U = 5 Z = 2.08 p = 0.04	U = 11 Z = 1.12 p = 0.31		U = 3 Z = 2.40 p = 0.02	U = 6 Z = 1.92 p = 0.06	U = 8 Z = 1.60 p = 0.13	U = 15 Z = -0.48 p = 0.70
	S. plana	Winter	U = 15 Z = -0.48 p = 0.70	U = 0 Z = -2.88 p = 0.00	U = 16 Z = 0.32 p = 0.82	U = 9 Z = -1.44 p = 0.18	U = 4 Z = 2.24 p = 0.03	U = 11 Z = -1.12 p = 0.31	U = 0 Z = -2.88 p = 0.00	U = 3 Z = -2.40 p = 0.02		U = 14 Z = -0.64 p = 0.59	U = 8 Z = -1.60 p = 0.13	U = 7 Z = -1.76 p = 0.09
		Summer	U = 17 Z = 0.16 p = 0.94	U = 1 Z = -2.72 p = 0.00	U = 11 Z = 1.12 p = 0.31	U = 16 Z = 0.32 p = 0.82	U = 2 Z = 2.56 p = 0.01	U = 16 Z = 0.32 p = 0.82	U = 9 Z = -1.44 p = 0.18	U = 6 Z = -1.92 p = 0.06	U = 14 Z = 0.64 p = 0.59		U = 18 Z = 0.00 p = 1.00	U = 8 Z = -1.60 p = 0.13
	S. marginatus	Winter	U = 12 Z = 0.96 p = 0.39	U = 1 Z = -2.72 p = 0.00	U = 10 Z = 1.28 p = 0.24	U = 17 Z = 0.16 p = 0.94	U = 2 Z = 2.56 p = 0.01	U = 15 Z = 0.48 p = 0.70	U = 9 Z = -1.44 p = 0.18	U = 8 Z = -1.60 p = 0.13	U = 8 Z = 1.60 p = 0.13	U = 18 Z = 0.00 p = 1.00		U = 11 Z = -1.12 p = 0.31
		Summer	U = 6 Z = 1.92 p = 0.06	U = 15 Z = -0.48 p = 0.70	U = 5 Z = 2.08 p = 0.04	U = 8 Z = 1.60 p = 0.13	U = 1 Z = 2.72 p = 0.00	U = 8 Z = 1.60 p = 0.13	U = 13 Z = 0.80 p = 0.48	U = 15 Z = 0.48 p = 0.70	U = 7 Z = 1.76 p = 0.09	U = 8 Z = 1.60 p = 0.13	U = 11 Z = 1.12 p = 0.31	
Ria Formosa lagoon	*C. edule*	Winter	U = 8 Z = -1.60 p = 0.13	U = 0 Z = -2.88 p = 0.00	U = 18 Z = 0.00 p = 1.00	U = 7 Z = -1.76 p = 0.09	U = 5 Z = 2.08 p = 0.04	U = 8 Z = -1.60 p = 0.13	U = 0 Z = -2.88 p = 0.00	U = 3 Z = -2.40 p = 0.02	U = 11 Z = -1.12 p = 0.31	U = 10 Z = -1.28 p = 0.24	U = 5 Z = -2.08 p = 0.04	U = 4 Z = -2.24 p = 0.03
		Summer	U = 4 Z = 2.24 p = 0.03	U = 10 Z = -1.28 p = 0.24	U = 1 Z = 2.72 p = 0.00	U = 1 Z = 2.72 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 1 Z = 2.72 p = 0.00	U = 7 Z = 1.76 p = 0.09	U = 16 Z = 0.32 p = 0.82	U = 0 Z = 2.88 p = 0.00	U = 4 Z = 2.24 p = 0.03	U = 4 Z = 2.24 p = 0.03	U = 15 Z = -0.48 p = 0.70
	C. gigas	Winter	U = 8 Z = -1.60 p = 0.13	U = 0 Z = -2.88 p = 0.00	U = 11 Z = -1.12 p = 0.31	U = 5 Z = -2.08 p = 0.04	U = 18 Z = 0.00 p = 1.00	U = 4 Z = -2.24 p = 0.03	U = 0 Z = -2.88 p = 0.00	U = 1 Z = -2.72 p = 0.00	U = 6 Z = -1.92 p = 0.06	U = 6 Z = -1.92 p = 0.06	U = 4 Z = -2.24 p = 0.03	U = 3 Z = -2.40 p = 0.02
		Summer	U = 4 Z = 2.24 p = 0.03	U = 8 Z = 1.60 p = 0.13	U = 3 Z = 2.40 p = 0.02	U = 5 Z = 2.08 p = 0.04	U = 1 Z = 2.72 p = 0.00	U = 4 Z = 2.24 p = 0.03	U = 6 Z = 1.92 p = 0.06	U = 6 Z = 1.92 p = 0.06	U = 3 Z = 2.40 p = 0.02	U = 4 Z = 2.24 p = 0.03	U = 5 Z = 2.08 p = 0.04	U = 10 Z = 1.28 p = 0.24
	M. galloprovincialis B**	Winter	U = 12 Z = -0.96 p = 0.39	U = 0 Z = -2.88 p = 0.00	U = 17 Z = 0.16 p = 0.94	U = 12 Z = -0.96 p = 0.39	U = 10 Z = 1.28 p = 0.24	U = 10 Z = -1.28 p = 0.24	U = 4 Z = -2.24 p = 0.03	U = 4 Z = -2.24 p = 0.03	U = 11 Z = -1.12 p = 0.31	U = 10 Z = -1.28 p = 0.24	U = 8 Z = -1.60 p = 0.13	U = 4 Z = -2.24 p = 0.03
		Summer	U = 0 Z = 2.88 p = 0.00	U = 5 Z = 2.08 p = 0.04	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 1 Z = 2.72 p =0.00	U = 2 Z = 2.56 p = 0.01	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 4 Z = 2.24 p = 0.03
	R. decussatus	Winter	U = 12 Z = -0.96 p = 0.39	U = 0 Z = -2.88 p = 0.00	U = 16 Z = 0.32 p = 0.82	U = 8 Z = -1.60 p = 0.13	U = 4 Z = 2.24 p = 0.03	U = 9 Z = -1.44 p = 0.18	U = 2 Z = -2.56 p = 0.01	U = 3 Z = -2.40 p = 0.02	U = 13 Z = -0.80 p = 0.48	U = 10 Z = -1.28 p = 0.24	U = 6 Z = -1.92 p = 0.06	U = 4 Z = -2.24 p = 0.03
		Summer	U = 3 Z = 2.40 p = 0.02	U = 14 Z = -0.64 p = 0.59	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 4 Z = 2.24 p = 0.03	U = 13 Z = 0.80 p = 0.48	U = 0 Z = 2.88 p = 0.00	U = 3 Z = 2.40 p = 0.02	U = 3 Z =2.40 p = 0.02	U = 18 Z = 0.00 p = 1.00
	S. marginatus	Winter	U = 6 Z = 1.92 p = 0.06	U = 1 Z = -2.72 p = 0.00	U = 3 Z = 2.40 p = 0.02	U = 6 Z = 1.92 p = 0.06	U = 0 Z = 2.88 p = 0.00	U = 5 Z = 2.08 p = 0.04	U = 17 Z = -0.16 p = 0.94	U = 9 Z = -1.44 p = 0.18	U = 1 Z = 2.72 p = 0.00	U = 10 Z = 1.28 p = 0.24	U = 11 Z = 1.12 p = 0.31	U = 11 Z = -1.12 p = 0.31
		Summer	U = 0 Z = 2.88 p = 0.00	U = 2 Z = 2.56 p = 0.01	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 4 Z = 2.24 p = 0.03
Total Protein Content (µg/g)			2643.25 ± 240.64	1278.88 ± 111.90	3197.62 ± 332.37	2625.11 ± 248.43	4076.92 ± 420.08	2634.06 ± 358.42	2016.67 ± 144.40	1785.44 ± 256.15	2835.31 ± 99.64	2576.88 ± 244.67	2439.15 ± 208.88	1724.49 ± 341.04

Appendix I. Results of the pairwise two-tailed Mann-Whitney U tests (U is the Mann-Whitney U-value, Z is the Z-value and p is the p-value) performed between the bivalve species sampled in both seasons, winter 2016 and summer 2017, and at both study areas, the Mondego estuary and the Ria Formosa lagoon, to estimate which groups have significant different ($p \leq 0.05$) distributions of total protein content (grey cells). Total protein content (mean ± standard error µg/g) of each sample is shown in the last row of the table. S* means small organisms; B** means big organisms. (cont.)

Study Area			**Ria Formosa lagoon**									
	Species		*C. edule*		*C. gigas*		*M. galloprovincialis* B**		*R. decussatus*		*S. marginatus*	
		Season	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Mondego estuary	*C. edule*	Winter	U = 8 Z = 1.60 p = 0.13	U = 4 Z = -2.24 p = 0.03	U = 8 Z = 1.60 p = 0.13	U = 4 Z = -2.24 p = 0.03	U = 12 Z = 0.96 p = 0.39	U = 0 Z = -2.88 p = 0.00	U = 12 Z = 0.96 p = 0.39	U = 3 Z = -2.40 p = 0.02	U = 6 Z = -1.92 p = 0.06	U = 0 Z = -2.88 p = 0.00
		Summer	U = 0 Z = 2.88 p = 0.00	U = 10 Z = 1.28 p = 0.24	U = 0 Z = 2.88 p = 0.00	U = 8 Z = -1.60 p = 0.13	U = 0 Z = 2.88 p = 0.00	U = 5 Z = -2.08 p = 0.04	U = 0 Z = 2.88 p = 0.00	U = 14 Z = 0.64 p = 0.59	U = 1 Z = 2.72 p = 0.00	U = 2 Z = -2.56 p = 0.01
	M. galloprovincialis S*	Winter	U = 18 Z = 0.00 p = 1.00	U = 1 Z = -2.72 p = 0.00	U = 11 Z = 1.12 p = 0.31	U = 3 Z = -2.40 p = 0.02	U = 17 Z = -0.16 p = 0.94	U = 0 Z = -2.88 p = 0.00	U = 16 Z = -0.32 p = 0.82	U = 0 Z = -2.88 p = 0.00	U = 3 Z = -2.40 p = 0.02	U = 0 Z = -2.88 p = 0.00
		Summer	U = 7 Z = 1.76 p = 0.09	U = 1 Z = -2.72 p = 0.00	U = 5 Z = 2.08 p = 0.04	U = 5 Z = -2.08 p = 0.04	U = 12 Z = 0.96 p = 0.39	U = 0 Z = -2.88 p = 0.00	U = 8 Z = 1.60 p = 0.13	U = 0 Z = -2.88 p = 0.00	U = 6 Z = -1.92 p = 0.06	U = 0 Z = -2.88 p = 0.00
	M. galloprovincialis B**	Winter	U = 5 Z = -2.08 p = 0.04	U = 0 Z = -2.88 p = 0.00	U = 18 Z = 0.00 p = 1.00	U = 1 Z = -2.72 p = 0.00	U = 10 Z = -1.28 p = 0.24	U = 0 Z = -2.88 p = 0.00	U = 4 Z = -2.24 p = 0.03	U = 0 Z = -2.88 p = 0.00	U = 0 Z = -2.88 p = 0.00	U = 0 Z = -2.88 p = 0.00
		Summer	U = 8 Z = 1.60 p = 0.13	U = 1 Z = -2.72 p = 0.00	U = 4 Z = 2.24 p = 0.03	U = 4 Z = -2.24 p = 0.03	U = 10 Z = 1.28 p = 0.24	U = 0 Z = -2.88 p = 0.00	U = 9 Z = 1.44 p = 0.18	U = 0 Z = -2.88 p = 0.00	U = 5 Z = -2.08 p = 0.04	U = 0 Z = -2.88 p = 0.00
	R. decussatus	Winter	U = 0 Z = 2.88 p = 0.00	U = 7 Z = -1.76 p = 0.09	U = 0 Z = 2.88 p = 0.00	U = 6 Z = -1.92 p = 0.06	U = 4 Z = 2.24 p = 0.03	U = 1 Z = -2.72 p =0.00	U = 2 Z = 2.56 p = 0.01	U = 4 Z = -2.24 p = 0.03	U = 17 Z = 0.16 p = 0.94	U = 0 Z = -2.88 p = 0.00
		Summer	U = 3 Z = 2.40 p = 0.02	U = 16 Z = -0.32 p = 0.82	U = 1 Z = 2.72 p = 0.00	U = 6 Z = -1.92 p = 0.06	U = 4 Z = 2.24 p = 0.03	U = 2 Z = -2.56 p = 0.01	U = 3 Z = 2.40 p = 0.02	U = 13 Z = -0.80 p = 0.48	U = 9 Z = 1.44 p = 0.18	U = 0 Z = -2.88 p = 0.00
	S. plana	Winter	U = 11 Z = 1.12 p = 0.31	U = 0 Z = -2.88 p = 0.00	U = 6 Z = 1.92 p = 0.06	U = 3 Z = -2.40 p = 0.02	U = 11 Z = 1.12 p = 0.31	U = 0 Z = -2.88 p = 0.00	U = 13 Z = 0.80 p = 0.48	U = 0 Z = -2.88 p = 0.00	U = 1 Z = -2.72 p = 0.00	U = 0 Z = -2.88 p = 0.00
		Summer	U = 10 Z = 1.28 p = 0.24	U = 4 Z = -2.24 p = 0.03	U = 6 Z = 1.92 p = 0.06	U = 4 Z = -2.24 p = 0.03	U = 10 Z = 1.28 p = 0.24	U = 0 Z = -2.88 p = 0.00	U = 10 Z = 1.28 p = 0.24	U = 3 Z = -2.40 p = 0.02	U = 10 Z = -1.28 p = 0.24	U = 0 Z = -2.88 p = 0.00
	S. marginatus	Winter	U = 5 Z = 2.08 p = 0.04	U = 4 Z = -2.24 p = 0.03	U = 4 Z = 2.24 p = 0.03	U = 5 Z = -2.08 p = 0.04	U = 8 Z = 1.60 p = 0.13	U = 0 Z = -2.88 p = 0.00	U = 6 Z = 1.92 p = 0.06	U = 3 Z = -2.40 p = 0.02	U = 11 Z = -1.12 p = 0.31	U = 0 Z = -2.88 p = 0.00
		Summer	U = 4 Z = 2.24 p = 0.03	U = 15 Z = 0.48 p = 0.70	U = 3 Z = 2.40 p = 0.02	U = 10 Z = -1.28 p = 0.24	U = 4 Z = 2.24 p = 0.03	U = 4 Z = -2.24 p = 0.03	U = 4 Z = 2.24 p = 0.03	U = 18 Z = 0.00 p = 1.00	U = 11 Z = 1.12 p = 0.31	U = 4 Z = -2.24 p = 0.03
Ria Formosa lagoon	*C. edule*	Winter		U = 0 Z = -2.88 p = 0.00	U = 11 Z = 1.12 p = 0.31	U = 2 Z = -2.56 p = 0.01	U = 16 Z = 0.32 p = 0.82	U = 0 Z = -2.88 p = 0.00	U = 16 Z = -0.32 p = 0.82	U = 0 Z = -2.88 p = 0.00	U = 0 Z = -2.88 p = 0.00	U = 0 Z = -2.88 p = 0.00
		Summer	U = 0 Z = 2.88 p = 0.00		U = 0 Z = 2.88 p = 0.00	U = 7 Z = -1.76 p = 0.09	U = 1 Z = 2.72 p = 0.00	U = 2 Z = -2.56 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 14 Z = -0.64 p = 0.59	U = 6 Z = 1.92 p = 0.06	U = 1 Z = -2.72 p = 0.00
	C. gigas	Winter	U = 11 Z = -1.12 p = 0.31	U = 0 Z = -2.88 p = 0.00		U = 0 Z = -2.88 p = 0.00	U = 13 Z = -0.80 p = 0.48	U = 1 Z = -2.72 p = 0.00	U = 9 Z = -1.44 p = 0.17	U = 0 Z = -2.88 p = 0.00	U = 1 Z = -2.72 p = 0.00	U = 0 Z = -2.88 p = 0.00
		Summer	U = 2 Z = 2.56 p = 0.01	U = 7 Z = 1.76 p = 0.09	U = 0 Z = 2.88 p = 0.00		U = 2 Z = 2.56 p = 0.00	U = 9 Z = -1.44 p = 0.18	U = 2 Z = 2.56 p = 0.01	U = 8 Z = -1.60 p = 0.13	U = 6 Z = 1.92 p = 0.06	U = 12 Z = -0.96 p = 0.39
	M. galloprovincialis B**	Winter	U = 16 Z = -0.32 p = 0.82	U = 1 Z = -2.72 p = 0.00	U = 13 Z = 0.80 p = 0.48	U = 2 Z = -2.56 p = 0.00		U = 0 Z = -2.88 p = 0.00	U = 14 Z = -0.64 p = 0.59	U = 1 Z = -2.72 p = 0.00	U = 5 Z = -2.08 p = 0.04	U = 0 Z = -2.88 p = 0.00
		Summer	U = 0 Z = 2.88 p = 0.00	U = 2 Z = 2.56 p = 0.00	U = 1 Z = 2.72 p = 0.00	U = 9 Z = 1.44 p = 0.18	U = 0 Z = 2.88 p = 0.00		U = 0 Z = 2.88 p = 0.00	U = 3 Z = 2.40 p = 0.02	U = 0 Z = 2.88 p = 0.00	U = 13 Z = 0.80 p = 0.48
	R. decussatus	Winter	U = 16 Z = 0.32 p = 0.82	U = 0 Z = -2.88 p = 0.00	U = 9 Z = 1.44 p = 0.17	U = 2 Z = -2.56 p = 0.01	U = 14 Z = 0.64 p = 0.59	U = 0 Z = -2.88 p = 0.00		U = 0 Z = -2.88 p = 0.00	U = 1 Z = -2.72 p = 0.00	U = 0 Z = -2.88 p = 0.00
		Summer	U = 0 Z = 2.88 p = 0.00	U = 14 Z = 0.64 p = 0.59	U = 0 Z = 2.88 p = 0.00	U = 8 Z = -1.60 p = 0.13	U = 1 Z = 2.72 p = 0.00	U = 3 Z = -2.40 p = 0.02	U = 0 Z = 2.88 p = 0.00		U = 2 Z = 2.56 p = 0.01	U = 2 Z = -2.56 p = 0.01
	S. marginatus	Winter	U = 0 Z = 2.88 p = 0.00	U = 6 Z = -1.92 p = 0.06	U = 1 Z = 2.72 p = 0.00	U = 6 Z = -1.92 p = 0.06	U = 5 Z = 2.08 p = 0.04	U = 0 Z = -2.88 p = 0.00	U = 1 Z = 2.72 p = 0.00	U = 2 Z = -2.56 p = 0.01		U = 0 Z = -2.88 p = 0.00
		Summer	U = 0 Z = 2.88 p = 0.00	U = 1 Z = 2.72 p = 0.00	U = 0 Z = 2.88 p = 0.00	U = 12 Z = 0.96 p = 0.39	U = 0 Z = 2.88 p = 0.00	U = 13 Z = -0.80 p = 0.48	U = 0 Z = 2.88 p = 0.00	U = 2 Z = 2.56 p = 0.01	U = 0 Z = 2.88 p = 0.00	
Total Protein Content (µg/g)			3086.84 ± 133.23	1660.41 ± 156.55	4626.82 ± 813.83	1275.65 ± 301.73	3293.88 ± 395.31	835.63 ± 124.38	2977.94 ± 145.57	1491.84 ± 149.15	2123.96 ± 99.35	884.56 ± 69.52

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