UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA ANIMAL

Aquaculture practices - a global characterisation and the case studies in the centre-south of Portugal revealing changes in species' nutritional composition

Carolina Vaz Vargas Pinheiro da Rocha

Mestrado em Ecologia Marinha

Dissertação orientada por:
Professor Doutor Henrique Cabral
Doutora Ana Marta Mendes Gonçalves

2017

Agradecimentos

Começo com um enorme agradecimento aos meus orientadores, por me terem dado a oportunidade de realizar o trabalho que aqui apresento, que, à medida que avançava, me foi despertando cada vez mais interesse.

À Doutora Ana Marta Gonçalves, muito obrigada por me ter acolhido ainda uma fresca aluna desconhecedora da beleza de serezinhos minúsculos, e me ter incentivado a seguir por este caminho, mesmo fugindo para os peixes. Por toda a disponibilidade e enorme apoio em todas as fases do trabalho, pela paciência para todas as minhas dúvidas e repetições distraídas, pela amizade.

Ao Professor Henrique Cabral por ter aceite esta orientação tão prontamente sem me conhecer (espero que as aulas não tenham entretanto trazido arrependimento!), pela total disponibilidade, incentivo e constante tranquilização. E um pedido de desculpas por todas as vezes que lhe bati à porta com pequenos pânicos que afinal não eram nada!

Gostaria de conseguir dizer-lhes o quanto agradeço o constante apoio mas uma certa incapacidade de expressão por escrito impede-me de vo-lo transmitir devidamente.

Ao Professor João Carlos Marques, pelo apoio logístico no trabalho desenvolvido.

Ao Professor Fernando Gonçalves, por todo o apoio logístico concedido. Ao grupo da Biologia, por me ter acolhido, sempre disponíveis para me ajudar no que fosse preciso.

À Doutora Cláudia Nunes pelo enorme apoio e disponibilidade durante todo este tempo, por todas as explicações e ajuda - aprendi imenso! Um grande agradecimento também a todo o grupo da Bioquímica por toda a simpatia e ajuda.

Um enorme agradecimento a todos os aquacultores que tão amavelmente me cederam o peixe para o trabalho, pela simpatia e pela disponibilidade para todos os esclarecimentos.

Ao Ricardo, pela paciência de me ensinar o que eu já devia saber e por não gozar comigo enquanto fazia aqueles pequenos lagos vermelhos – um dia hei de conseguir!

À Filipa por ter estado sempre presente, por ter rido os meus momentos de médio stress, por todas as dúvidas de meia-noite!

À Doutora Ana Cristina Falcão por todo o acompanhamento no trabalho no Sado, pela boa disposição e ajuda, pela prontíssima resolução de todas as dúvidas que foram surgindo nas alturas mais estranhas.

À Doutora Célia, obrigada por ter andado comigo de “um lado para o outro” em busca do material precisava e pela total disponibilidade para o que precisasse.

À Doutora Vanessa Fonseca, obrigada pelas óptimas sugestões e respostas tão rápidas às dúvidas que me foram surgindo.

Aos CdM (arranjei-nos também uma sigla amigos!) Sofs, Rita, Tilde, Keat, Ritinha, Ricardo, Gui, André, Vasco (que me ouviram ziliões de vezes, que vão fazer-me uma festa mesmo que me espalhe ao comprido – é muito estranho já não vos ter a todos ali ao lado a qualquer hora), à Raquel, às donzelas Pris, Sheila e Inês (por toda a ajuda, preocupação e companhia no stress, são umas lindas), à Catarina e Joana (foi tudo tão mais fácil assim), à Sófia (as vezes que me ouviste, sei que secretamente tinhas

preferido que fossem sardinhas!), à Ju, Laura e Mimi (fazem-me falta meus Bois), enfim, aos meus amigos, que levo para a vida, que me ouviram, acalmaram, ajudaram, incentivaram e que foram companheiros de stress também!, a quem não consigo agradecer porque não é possível explicar.
Ao Bernardo, a quem perguntei milhões de vezes opinião sobre a mesma coisa e fez como se estivesse a ouvir pela primeira vez (quase sempre! eheh), que impediu que desfalecesse de fome quando sentia que não havia tempo para nada que não fosse estar agarrada ao computador, por todo o carinho e amor nas alturas mais terríveis (sim, sim, não sei ser muito linda a escrever mas sabes o que quero dizer).

Aos meus pais, que desde sempre fizeram com que tudo fosse muito possível, que me ensinaram que a vida não espera por mim para acontecer, que sempre abriram caminho para que fosse eu a decidir o rumo a dar à minha vida. E por todos os conselhos e opiniões que pareci desmerecer, sabem que têm só que me ignorar (os filhos têm que ter estes momentos irritantes às vezes, faz parte da experiência! (?) eheh)

Aos meus avós, que são pais duas vezes, que me ouviram vezes sem conta falar do mesmo, sempre com interesse, fazendo sempre com que pensasse um pouco mais além. Não teria sido possível ultrapassar os momentos mais difíceis (normais destas coisas, espero) sem a vossa ajuda, válido também para o caminho que se segue! Sei que o sabem.

Ao meu irmão que me fez fazer uma pausa quando estava prestes a assassinar o computador (e mesmo que tenha parecido que a ti também era tudo a fingir), que é o meu revisor super eficaz e companheiro de trabalho enquanto o resto do mundo está ainda na praia.

Mas à família não se agradece, não é sequer possível.

A todos aqueles que foram tão importantes ao longo deste trabalho e na minha vida, que, mesmo não sendo aqui referidos, foram determinantes para que tudo fosse possível.

Resumo

O crescimento exponencial verificado na população humana mundial tem vindo a colocar diversas questões acerca da capacidade do planeta em fornecer recursos que suportem a crescente procura de bens e serviços. A preocupação dominante na actualidade será tanto se existirá disponibilidade de recursos alimentares em quantidade suficiente para uma população cada vez mais numerosa, como os processos de obtenção desses recursos e consequentes impactes ambientais.

O aumento da produção de alimento de origem marinha produz impactes de grande magnitude nas populações selvagens marinhas, que são frequentemente sobreexploradas e mal geridas; a preocupação com o estado ecológico do ambiente e das populações selvagens desencadeou também o desenvolvimento da aquacultura, com principal expressão a partir dos anos 70, um pouco por todo o mundo. Começando como actividade complementar à pesca, por forma a ser possível satisfazer a procura de pescado sem aumentar a pressão sobre os *stocks* de pescado selvagem, a produção aquícola foi assumindo uma dimensão e importância crescentes ao longo dos anos, passando a constar de muitos programas de desenvolvimento económico. Em resultado da expansão desta actividade, o volume de pescado destinado ao consumo humano produzido em aquacultura ultrapassou, em 2013, a quantidade daquele produto capturada pela actividade pesqueira. A Ásia tem sido, nas últimas décadas, o principal produtor de espécies aquáticas. Com efeito, o continente asiático foi responsável, em 2014, por 89% do total mundial de produção aquícola, sendo que a China, por si só, produziu naquele ano cerca de 62 % do volume total produzido em aquacultura mundialmente. A Europa, por seu turno, ocupava em 2014 o terceiro lugar do ranking de produção aquícola no mundo, correspondente a 4% do total de organismos aquáticos produzido em 2014, sendo a Noruega o seu maior produtor, com cerca de 50% do total europeu.

Apesar da longa tradição pesqueira de Portugal, há muito que a aquacultura é praticada no país, ainda que em pequena escala. Em Portugal, a produção aquícola centra-se essencialmente na produção de espécies marinhas, principalmente em sistemas de transição, como estuários e lagoas costeiras, utilizando métodos extensivos e semi-intensivos de produção. A produção de espécies de peixes marinhos correspondeu, em 2014, a cerca de 48% do total da produção aquícola nacional e, apesar de os sistemas semi-intensivos serem os mais comuns para a produção de peixe marinho, o volume de produção das aquaculturas em sistema intensivo ultrapassa, desde 2010, o volume de produção de peixe em sistemas semi-intensivos. No entanto, apenas o pregado (*Scophthalmus maximus*) é produzido intensivamente.

O facto de muitas espécies marinhas, como a dourada (*Sparus aurata*) e o robalo (*Dicentrarchus labrax*), estarem bem adaptadas às condições ambientais de sistemas de transição, uma vez que os utilizam comummente como zonas de reprodução, alimentação e abrigo durante as primeiras fases de desenvolvimento, permite o seu cultivo nesses sistemas. A dourada e o robalo foram, respectivamente, a segunda e terceira espécies marinhas mais produzidas em Portugal em 2014.

As unidades de produção semi-intensiva em estuários utilizam a água do sistema para o enchimento dos tanques de peixe, controlando a sua entrada e saída conforme as necessidades. O peixe é alimentado artificialmente, com recurso a rações que permitem ao produtor controlar o fornecimento de nutrientes que confiram ao produto a composição desejada. Este método de produção é, no entanto, pouco controlado, uma vez que as características da água nos tanques dependem da qualidade da massa de água envolvente. Por outro lado, não é possível controlar com total precisão a alimentação dos peixes em sistemas semi-intensivos, uma vez que a água conduzida para os tanques de produção poderá ser portadora de organismos e matéria orgânica de que os peixes poderão alimentar-se. O desenvolvimento de peixes depende, assim, não só das suas características intrínsecas, geneticamente determinadas, mas

também das características dos tanques de crescimento (e.g. dimensão), dos factores ambientais e da qualidade da água. Deste modo, a mesma espécie produzida em regime semi-intensivo em sistemas estuarinos distintos poderá ter um desenvolvimento diverso que se traduzirá, previsivelmente, em diferente conteúdo nutritivo resultante da acção conjunta de todos estes factores (alimento, stress no tanque, água, parâmetros ambientais) ao ativarem genes (alelos) diferentes, que podem potenciar uma maior ou menor produção nutricional.

O peixe é considerado um alimento saudável, principalmente devido ao seu conteúdo em proteína de elevada qualidade, fonte de minerais e outros nutrientes essenciais, e por constituir a principal fonte de ácidos gordos altamente insaturados para o ser humano, destacando-se o seu conteúdo em ácidos gordos ómega-3, com acção de prevenção de doenças cardiovasculares e autoimunes e necessários ao desenvolvimento e funcionalidade do cérebro e retina.

O presente trabalho, além de realizar uma revisão sobre a situação e tendências da aquacultura em diferentes contextos geográficos, analisou o conteúdo nutritivo em robalos e douradas provenientes de aquaculturas em regime semi-intensivo em dois estuários portugueses (Mondego e Sado). Para a caracterização do perfil nutritivo dos indivíduos, foram recolhidos robalos e douradas no final do ciclo de produção em quatro aquaculturas, duas por estuário. Foram recolhidas amostras de tecido muscular de três indivíduos de cada espécie recolhida em cada uma as aquaculturas para cada análise bioquímica, tendo a quantificação de proteína total sido realizada segundo o método descrito por Bradford (1976), a análise do perfil em ácidos gordos por cromatografia gasosa-espectrofotometria de massa (GC-MS) e a análise de açúcares livres e polissacarídeos através de cromatografia gasosa com detector por ionização de chama (GC-FID). As principais diferenças entre as quantidades dos macronutrientes presentes nos peixes foram avaliadas através de análises de variância (ANOVA); foi ainda efectuada uma análise de componentes principais (PCA) para evidenciar os perfis em ácidos gordos dos indivíduos de ambas as espécies das diferentes aquaculturas dos dois sistemas estuarinos.

A análise do conteúdo proteico não revelou diferenças significativas entre organismos de diferentes origens e a concentração de proteína total no tecido muscular dos peixes analisados mostrou ser superior ao estimado para indivíduos selvagens da mesma espécie.

Relativamente à análise de ácidos gordos, verificaram-se diferenças significativas quanto ao conteúdo total de ácidos gordos saturados, monoinsaturados e altamente insaturados em robalos entre os dois estuários e entre as aquaculturas de cada estuário; em dourada foram encontradas diferenças quanto ao conteúdo em ácidos gordos saturados, monoinsaturados, poli-insaturados e altamente insaturados apenas entre estuários. A avaliação de diferenças entre ácidos gordos com maior importância para a saúde humana verificou diferenças significativas entre estuários para o conteúdo em ácido eicosapentaenóico, ácido docosahexaenóico, ácido araquinóico e ácido linoleico em ambas as espécies e apenas no conteúdo dos ácidos eicosapentaenóico e araquinóico entre aquaculturas do mesmo estuário em robalos. De um modo geral, as duas espécies criadas no estuário do Sado apresentaram maior conteúdo em ácidos gordos em relação aos organismos produzidos nas aquaculturas no estuário do Mondego.

O perfil em hidratos de carbono das espécies estudadas analisou o conteúdo em açúcares livres e polissacarídeos. O açúcar livre mais abundante nas duas espécies foi a glicose, seguindo-se o conteúdo em arabinose, tendo sido observadas diferenças significativas quanto ao conteúdo dos dois açúcares entre estuários, observando-se maior quantidade nos organismos produzidos nas aquaculturas do Mondego; verificou-se ainda a presença de manose apenas em organismos produzidos no estuário do Mondego, nas duas espécies de peixe.

Relativamente ao conteúdo em polissacarídeos, a glicose foi o monómero encontrado em maior quantidade nas duas espécies, seguido pelo conteúdo em arabinose; manose e galactose foram encontradas em reduzidas quantidades e apenas em organismos produzidos nas aquaculturas do Mondego, em ambas as espécies de peixes.

As diferenças nutricionais identificadas no presente estudo permitiram verificar que o local de produção teve influência na composição nutritiva de uma mesma espécie, pelo que os factores ambientais, a qualidade da água, influenciada pelo potencial diferente i*nput* de nutrientes e poluentes por fontes antropogénicas localizadas e difusas, o regime alimentar e o stress provocado pela densidade de indivíduos nos tanques de produção, a que os organismos estão sujeitos durante o seu desenvolvimento, poderão estar na origem dessas diferenças.

A continuidade do estudo desenvolvido no sentido de apurar quais as características do meio que estarão a causar as diferenças encontradas, permitirá aprofundar o conhecimento acerca dos limites das técnicas de regime semi-intensivo para produção aquícola e a forma como poderão ser melhoradas e adaptadas, por forma a corrigir essas limitações. Ainda, o contínuo aumento da produção de pescado em sistemas aquícolas tem vindo a levantar preocupações acerca do balanço entre os benefícios trazidos pelo consumo de peixe e o seu eventual grau de toxicidade. Deste modo, considera-se que seria interessante avançar para uma segunda etapa do estudo realizado com a análise da contaminação dos organismos produzidos, e se um consumo prolongado destes produtos poderá trazer riscos à saúde humana.

Palavras-chave

Aquacultura, produção global, sistema semi-intensivo, caso de estudo de Portugal, conteúdo nutritivo.

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Summary

The overfishing of most wild fish stocks with commercial interest and the environmental concerns it raises have, especially since the 1970's, fomented the development of aquaculture worldwide. Asia has for decades maintained its status as the globe's main aquaculture producer, accounting for almost 90% of the world's total aquaculture production in 2014; Europe occupied the third place in terms of volume of production in the same year, contributing with about 4% of the total world production in aquaculture. Portugal is a traditionally fishing country which has yet poorly developed its aquaculture production, focusing mainly on the rearing of marine species, especially fish, whose production accounted for 48% of the total national production (including freshwater and marine species) in 2014. The main reared species in Portugal in 2014 was the turbot (*Scophtalmus maximus*), in intensive rearing systems; however, such systems are aimed essentially for the rearing of that particular species, and most marine fish species are reared in semi-intensive systems in transitional water systems, such as estuaries and coastal lagoons along the country's coast. Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) were, respectively, the second and third most reared fish species in Portugal in 2014, cultured mainly in facilities in estuaries, using semi-intensive production systems.

Fish development and consequent nutritional value are dependent on the intrinsic factors of the organisms, which are genetically determined, and on the extrinsic factors to which the fish are subjected to during their life cycle. There is poor control over the environmental factors in semi-intensive production systems, essentially due to the fact that, although feed is provided by the farmer, controlling to some extent the products' nutritional intake, the water used in the rearing ponds comes from the main water system in which the production facilities are placed in, which may bring small organisms and nutrients, in which the fish may also feed on, and pollutants to the ponds. Environmental factors, water quality, feeding regimes and stress related to fish density within the rearing ponds may also be distinct among different systems, and thus the same species reared in semi-intensive systems may present different nutritional composition depending on the production site's characteristics.

Gilthead seabream and European seabass specimens reared in four different aquacultures in two Portuguese estuaries (Mondego and Sado) were studied with the purpose of assessing if the rearing site influenced the nutritional value of conspecifics reared in distinct aquacultures. Analyses of total protein content and fatty acid and carbohydrate profiles were carried out using the muscle tissue of individuals from each aquaculture, and the differences in each macronutrient content in the fish species studied were assessed by analysis of variance (ANOVAs); a principal component analysis was also carried out in order to characterize the fatty acid profile of both species from each aquaculture in the two estuaries.

The analysis of protein content in fish muscle tissue showed no differences among the groups studied of both species, either between conspecifics from different estuaries or between the two aquacultures within each estuary.

Fatty acid content results, however, presented some differences between the groups analysed, with fish of both species reared in the aquacultures in the Sado estuary presenting an overall higher content of total saturated, monounsaturated, polyunsaturated and highly unsaturated fatty acids compared to their conspecifics reared in the aquacultures in the Mondego estuary. Analysis of eicosapentaenoic, docosahexaenoic, arachidonic and linoleic acids content also showed differences in the two species studied, especially between fish among the two estuaries, with fish reared in the aquacultures in the Sado estuary presenting an overall higher content of the named fatty acids.

The analysis of carbohydrate profile comprehended the assessment of free sugars and polysaccharides content in samples, the latter determined based on the content of the monosaccharides that constitute the polysaccharides. Free sugar analysis showed that glucose was the most abundant sugar in fish tissue, followed by arabinose; both sugars presented differences in conspecifics among estuaries for both species, with seabass and seabream reared in the aquacultures in the Mondego estuary presenting higher

content than their conspecifics reared in the Sado estuary. Mannose was only found in fish reared in the aquacultures on the Mondego estuary for both species, although in considerably low concentrations. Polysaccharide analysis found glucose to be the most abundant monomer, and significant differences were observed in its content in fish between estuaries for both species. Mannose and galactose monomers were only found in fish reared in the aquacultures in the Mondego estuary, although in very low concentrations.

The differences found on the nutritional content between conspecifics reared in distinct aquacultures allowed to verify that the rearing site, and thus its dominant environmental characteristics, influences the development of the aquaculture products cultured in semi-intensive systems, which present different organoleptic compositions when commercialized. From the production view, the influence of factors that cannot be controlled by the farmer on the products' quality may come as a disadvantage for this rearing method.

It would be interesting in the future to develop the present study in order to clearly identify and assess the impact of the factors that may be on the origin of the differences found, enriching the knowledge about this type of rearing system's limitations and eventually contribute to their minimization. Regarding the ever-growing aquaculture production, questions about the quality of the products in terms of potential contamination have been raised; estuaries are areas subjected to various human pressures, with high pollutant input from many different sources, and thus it would be interesting to assess the fish pollutant content from the studied aquacultures, and understand its possible relation with the pollutants in the environment, in order to assess if a long-term exposure to these fish may pose health risks to the consumers.

Key-words

Aquaculture, global production, semi-intensive system, Portugal case study, nutritive composition.

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Chapter 1

General Introduction

The exponential growth of human population in the last decades has led to a variety of issues regarding food exploitation and supply, both if there will be enough food resources to support that ever-growing number and if the way those products are exploited and distributed will attend to the needs in the future (MEA, 2005; Pauly, Watson, & Alder, 2005).

Global demand for fish supply has been rising over the years (FAO, 2016), not only due to the above-mentioned population growth, but also due to the recognition of the health benefits that fish consumption may bring to its consumers (Harris, Kris-Etherton, & Harris, 2008; Harris, Poston, & Haddock, 2007; IOM, 2011; Lee et al., 2009; Russo, 2009; Simopoulos, 2009), although concerns about fish quality and safety for consumption have also been raised. Years of unsustainable exploiting techniques to match such demands have resulted in negative impacts on wild fish stocks, with most of them currently overexploited, if not depleted (Mora et al., 2009).

Aquaculture had been kept as a secondary animal protein production activity until recently, and has proven to be a profitable practice. However, fisheries present great economic importance, especially in coastal countries with a long fishing tradition, as is the case of Portugal, where fisheries make up a considerable portion of the country’s economy and sustain great cultural value, which, associated with differences between sensory properties of wild and farmed fish may pose constraints against aquaculture development.

Moreover, aquaculture also poses some threats to the surrounding environment: commercial feeds for cultured fish contain fish meal and fish oils that need raw material to be produced, obtained through fisheries of wild stocks (Izquierdo et al., 2003; Sargent & Tacon, 1999) which, instead of releasing pressure over wild populations, upholds it (FAO, 2016; Simard, Ojeda, & Haroun, 2008). The areas occupied by aquaculture facilities can easily extend largely, destroying natural habitats and the landscape, which reinforces the need for a careful site selection, to minimize potential threats from the interaction between aquaculture facilities and the surrounding environment. Aquaculture farms can be sources of high nutrient input and contamination of the surrounding environment through effluent discharges, usually containing food, fish excretions and remains, drugs used to enhance production, among others, all of which contribute to potentially lower the quality of the surrounding water bodies (Simard, Ojeda, & Haroun, 2008). However, this particular issue can be easily solved if the right waste management is applied in the aquaculture facility and the site for production is well-suited. Should farmed organisms escape form the rearing farms, they may pose a threat to wild populations’ genetic pool, introduce parasites and diseases, exotic species may be introduced in the surrounding environments or serve as predators to local species; in the case of bivalve molluscs, when reared in bottom cultures, they may modify deeply the bottom structure and affect plankton food chains.

Therefore, a balance between environmental costs and benefits of aquaculture production must be found. A thorough local planning of the production, from spatial planning to the rearing methods used, defining strategies to enhance productivity and diminish environmental impacts, should be present in every country’s agenda for aquaculture development. From a local strategy, a global plan should also be defined, in order to adequate production to the specificities of the different areas, searching for a global balance in this activity. As the development of aquaculture is considerably recent in many countries, strategic plans and programs should be developed and implemented, not only locally but to a continental level, which will require a great amount of work from aquaculture farmers, authorities, stakeholders and other entities involved.

Although fish supply for human consumption from aquaculture has already surpassed that of fisheries, concerns about farmed fish quality have been raised amongst the general public and many studies have been conducted to assess the differences between wild and farmed fish. Potential drug’s usage to promote growth, differences in texture, taste and aroma from wild fish are amongst the main concerns raised. Fortunately, spreading of such concerns has also resulted on the honing of aquaculture methods and practices, especially concerning the control of water quality and feeding of farmed organisms, searching for higher product quality.

To approach such a complex situation, it was thought to be important to give an insight on aquaculture's global development over the years, with greater focus on the development of aquaculture in Continental Portugal, over the period between 1999 and 2014. Aquaculture in Portugal is mainly focused on the rearing of marine species, which has for long been carried out in transitional waters (estuaries and coastal lagoons) along coastal areas. Although recent developments have expanded the production of fish in intensive systems, these focus mainly on the rearing of a single species. Thus, semi-intensive and extensive systems are the most common production systems used for fish production, as it is common in countries with yet small aquaculture methods developed. Since the water used in aquaculture tanks comes from the surrounding aquatic systems, it raises the concern that water quality together with other factors, such as environmental parameters, stress in the rearing tanks, food quality and quantity, among other factors, may influence fish development and potentiate different nutritive compositions when at a marketable size.

The present work will then comprehend two chapters addressing the subjects above mentioned: one chapter will address the current status of aquaculture production worldwide, providing a more detailed insight of the evolution of aquaculture in Continental Portugal between the years 1999 and 2014; the other chapter will focus on the characterization of the nutritive composition of fish species (*Dicentrarchus labrax* and *Sparus aurata*) reared in two Portuguese estuaries (Mondego and Sado), subjected to different sets of extrinsic factors potentially influencing differently their development.

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Chapter 2

The current status of aquaculture in the world with a focus on the activity's development in Portugal

2.1. Introduction

Aquaculture is, according to Food and Agriculture Organization of the United Nations (FAO), “(...) the farming of aquatic organisms in both coastal and inland areas involving interventions in the rearing process to enhance production.”, an activity that, although dating back thousands of years, has only recently began to experience great development.

It is unclear when aquaculture began but findings over the years show that this activity has been carried out for centuries, all around the globe. It is suggested that the first steps in aquaculture production may date back to the Neolithic age, around 4000 B.C., consisting on trapping aquatic animals in small waterbodies in order for them to be available at any time needed. Pictorial engravings on ancient Egyptian tombs, dating from 2500 B.C., represent the harvest of tilapia from artificial ponds; the rearing of carps in China, dating back to the fifth century B.C., is probably the most well-known evidence of ancient aquaculture methods. In Europe, Romans fattened fish in tanks that were aimed for the rearing of oysters or for salt production, but it was only during the Middle Ages that aquaculture in Europe started to develop, where fish, specially carps, were cultured in ponds in monasteries (Dinis et al., 1999; EC, 2017).

Seafood farming in brackish waters also began to develop around that time in Southern European countries, where coastal lagoons and ponds were used to maintain finfish brought in by the ocean tide, usually alternating with salt production in the same areas (EC, 2017).

As for the farming of molluscs, it began to be developed in the 13th century in pole cultures, especially for the production of oysters and mussels, spreading along the French Atlantic coastline during the 19th century; Northern European countries, on the other hand, developed bottom cultures for the rearing of the same organisms (EC, 2017).

Aquaculture worldwide experienced a significant development in the beginning of the 1900’s and again around the 1970’s, when awareness of the negative impacts that years of intensive fishing caused on wild fish stocks raised an ecological concern, which contributed to the development of aquaculture activity and discussion about its benefits and importance in either ecological and economical perspectives. Hence, around that time, aquaculture practices began to be regarded not only as a means to obtain seafood complementary to capture fisheries, but also as a way of releasing the overpressure on wild stocks and a means to produce organisms that could be used to restock overexploited stocks.

The development of fisheries and aquaculture enabled populations to search for more diversified diets which, together with the awareness of the health benefits associated to seafood consumption, lead to an increase in the demand for such products, hence stimulating the development of the aquaculture activity. According to FAO, seafood production from capture fisheries has stabilized over the last two decades, whereas aquaculture production has increased, almost hand-to-hand with the growth in seafood consumption worldwide (FAO, 2016).

The volume of aquaculture’s products surpassed that of fisheries in 2013; in what concerns to seafood supply for human consumption, aquaculture surpassed the supply from capture fisheries for the first time in 2014 (FAO, 2016). The total aquaculture seafood production in the world in the year 2014 accounted for about 73.8 million tonnes, valuing an estimate of 160.9 billion US dollars, being fish the main cultured group, valuing about 99.0 billion US dollars (Figure 2.1.1) (FAO, 2016).

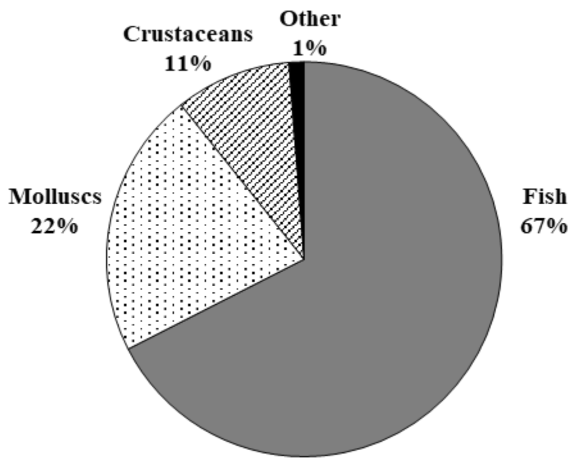

Figure 2.1.1 - Production of main seafood groups: percentage of the groups' production in terms of volume, concerning the total aquaculture production in 2014 in the world. Source: FAO, 2016.

The consumption of seafood *per capita* has increased nearly 10 kg since the 1960's (FAO, 2016) and it is estimated that aquaculture products' contribution for the world's seafood supply has been increasing about 8.8% per year since 1970 (DGRM, 2017). Aquaculture production of fish grew at a rate of 5.8% annually in the decade 2005-2014 (FAO, 2016), with inland aquaculture representing about 65% of that increase, as it is the most common type of aquaculture in the world (FAO, 2016). The world production of fish in marine and coastal waters in 2014 was about 26.7 million tonnes, around 36.2% of the total aquaculture seafood production (Figure 2.1.2) (FAO, 2016).

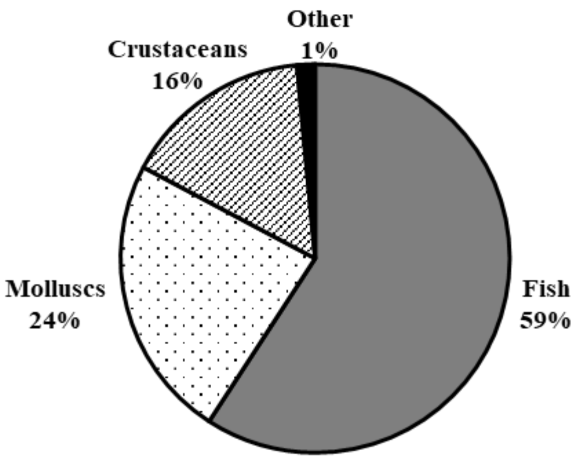

Figure 2.1.2 – Main seafood groups' production in marine and coastal waters by volume: percentage of total aquaculture production in marine and coastal waters in 2014 worldwide. Source: FAO, 2016.

2.2. A global overview

The status of aquaculture production worldwide is revised in the present work, based on the available data reporting to the year 2014, with a view on the aquaculture production and practices in the three main producing continents, Asia, America and Europe.

Asia is the leading continent in marine, estuarine and freshwater seafood aquaculture production, both in terms of volume and estimated first-sale value, producing in 2014 about 89% of the total volume of aquaculture products worldwide, worth close to 77% of the total income from aquaculture products' sale worldwide. America is the second continent with the highest production in terms of volume, accounting for 5% of the total aquaculture production, valuing about 12% of the world's aquaculture earnings that year, and followed by Europe, which contributed for about 4% of production and with over 8% of the total income from aquaculture products. Africa produced about 2% of the world's farmed aquatic animals in 2014, which were worth a similar percentage in terms of value, and Oceania accounted for a production of less than 1% of the total world aquaculture production in volume in 2014, valuing, however, about 1% of the global earnings form aquaculture production (FAO, 2016; NMFS, 2016). Although Asia is on the lead of aquaculture production concerning both volume and value, the products farmed especially in America and Europe sustain greater market value per volume unit, although that may not be clear when comparing total volume or value numbers. The contribution of the different types of aquaculture in each continent total production is represented in Figure 2.2.1.

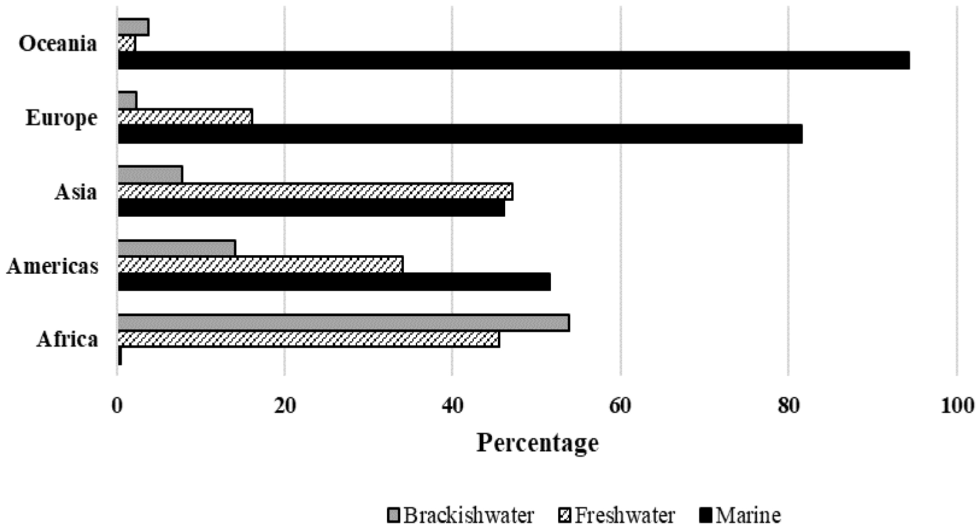

Figure 2.2.1- Seafood production in brackish, fresh and marine aquaculture: percentage of total production in 2014 by system in each Continent. Source: FAO, 2016.

Asia has led the aquaculture production worldwide for the past two decades, with the five main aquaculture producing countries in the world being in Asia: China has for long been the main producer in the world, accounting, by 2014, for about 45.5 million tonnes of aquaculture products, about 61.7% of the world's total aquaculture production, followed by India (6.6%), Indonesia (5.8%), Viet Nam (4.6%) and Bangladesh (2.7%) (FAO, 2016; NMFS, 2015).

Freshwater aquaculture is the most common type of aquaculture in Asia, representing about 66.5% of the Continent's aquaculture production. Nonetheless, Asia is on the lead of marine and coastal aquaculture in the world as well, accounting, by 2014, for 21.8 million tonnes of seafood production, about 81.7% of the world's total marine and coastal aquaculture production (FAO, 2016).

Fish is the main group produced in Asia, especially freshwater fish (Figure 2.2.2); grass carp is the main produced species in the Continent, followed by other carp species such as bighead carp and common carp, followed by molluscs, being clams and cockles the main mollusc groups produced, and oysters the third main species produced. The contribution of the main seafood groups for aquaculture production of the five main Asian countries is represented in figure 2.2.3.

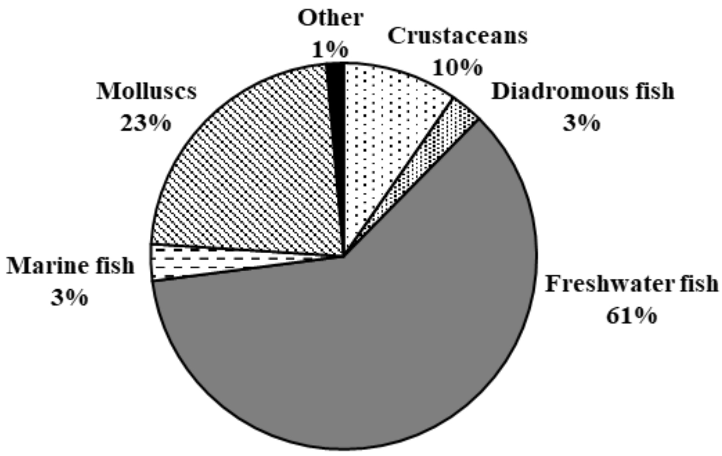

Figure 2.2.2 – Aquaculture production of the main fish groups in Asia: percentage of the total Asian volume of aquaculture fish production in 2014. Source: FAO, 2016.

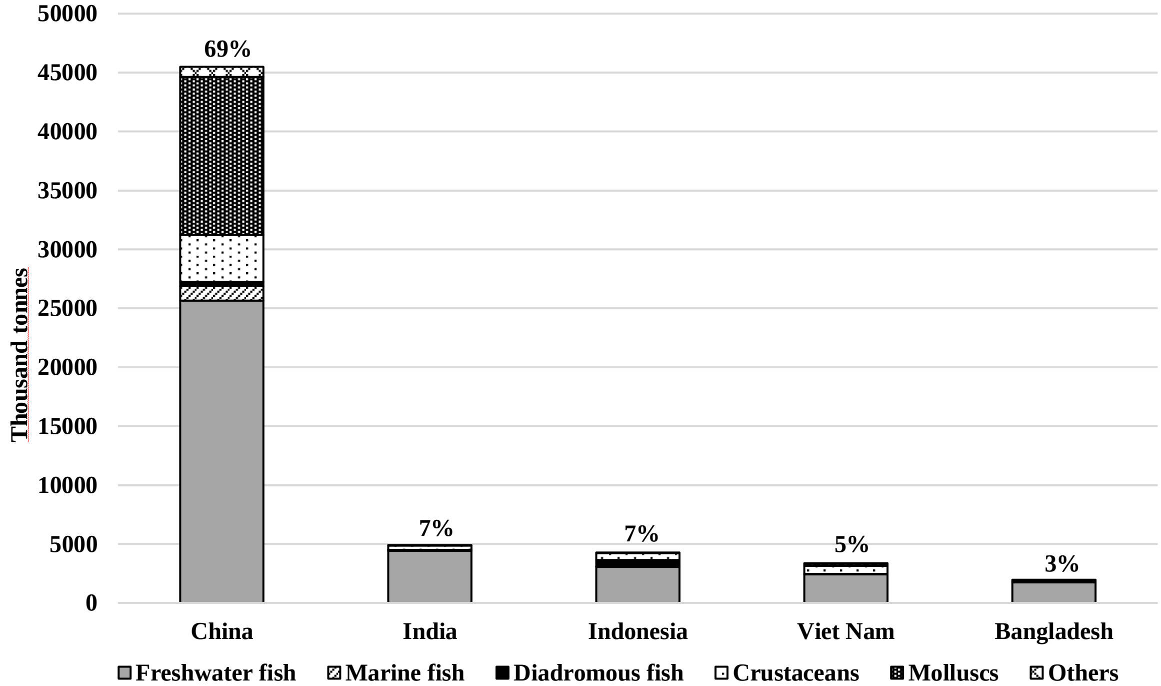

Figure 2.2.3 - Production by volume of main fish groups in the main producing countries in 2014. Values above each column represent the aquaculture production of each country in terms of percentage of the total Asian aquaculture production in 2014.

America is the second main producing Continent in terms of aquaculture production volume, accounting for the production of about 5% of the total aquaculture production worldwide in 2014. Aquaculture in America focuses mainly on the rearing of species in marine waters (about 52% of the total aquaculture production), and the contribution of each seafood group for the Continent's total production is represented in Figure 2.2.4; the main species reared are Atlantic salmon, representing about 22.4% of the total production and whiteleg shrimp representing about 19% of the production, both reared mainly in marine and coastal environments. Chile is the major producer in the Continent, accounting for about 36. 3% of the total American production; and is as well the main producer of Atlantic salmon in the Continent and the second worldwide; Brazil, the United States of America and Ecuador followed as main producing countries. Canada, although not a main producing country in terms of volume, is the second most important producer of Atlantic salmon in the Continent and the fifth worldwide (Figure 2.2.5) (FOC, 2015, 2016).

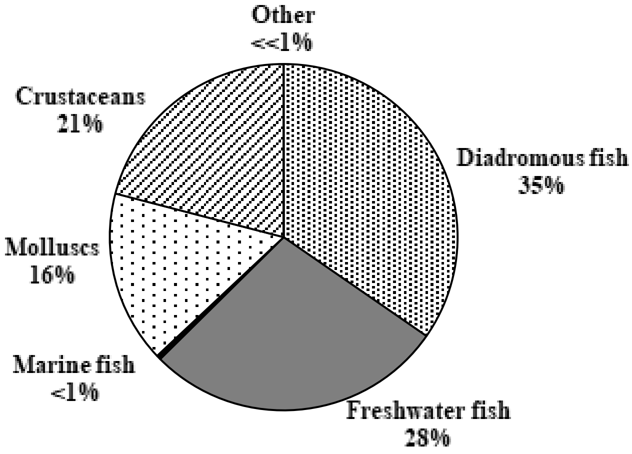

Figure 2.2.4 – Aquaculture production of the main groups in America: percentage of total volume of production in 2014. Source: FAO, 2016.

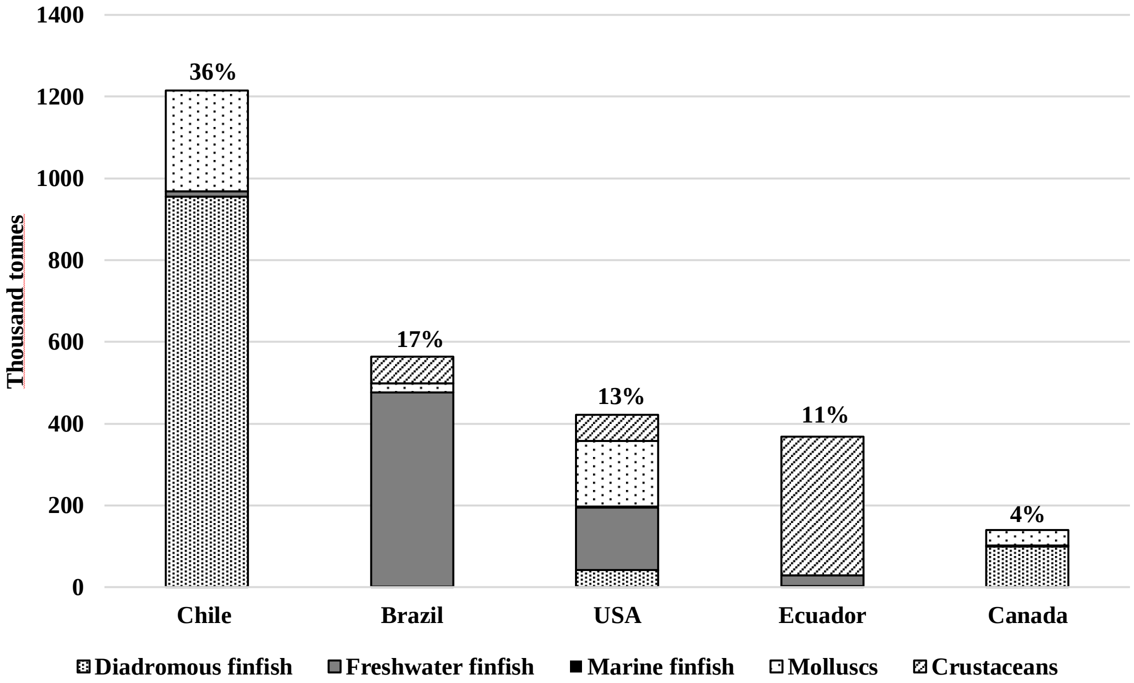

Figure 2.2.5 – Production by volume of main fish groups by main producing country in America in 2014. Values above each column represent the aquaculture production of each country in terms of percentage of the total American aquaculture production in 2014. Source: FAO, 2016.

The aquaculture production in Europe in 2014 represented about 3% of the world's aquaculture production, with an output of about 2.9 million tonnes.

Marine species make up most of the European aquaculture, accounting, by 2014, for about 84% of the Continent's total production (FEAP, 2015), thus leading most of European aquaculture to be carried out in marine and coastal facilities.

Fish and molluscs were the main species' group reared, representing nearly the whole of the aquaculture production in Europe in 2014; the production of crustaceans and other animals represented a minor slice of Europe's production (Figure 2.2.6).

Norway is the leading European country in aquaculture production, with an output of almost 50% of the total production in Europe by volume in 2014 (about 1.33 million tonnes of live weight) and the sixth main producer worldwide (EUMOFA, 2016; FEAP, 2015).

Spain, the United Kingdom and France were the following top producers of the European Union (EU) member-states, accounting, together, for about 24% of the total European production in 2014 (EUMOFA, 2016). The contribution of each species' group for the aquaculture production in the main European producing countries is represented in Figure 2.2.7.

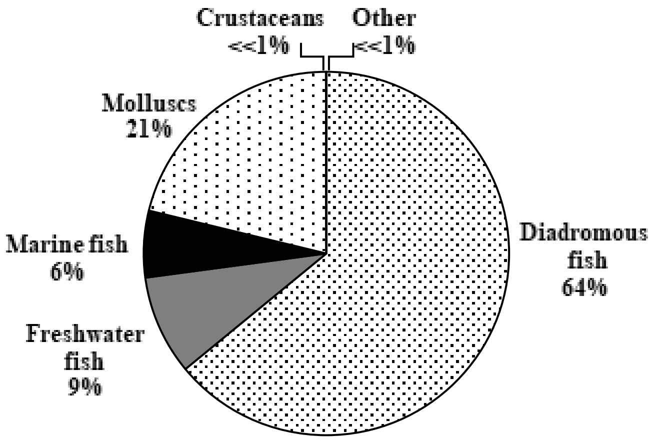

Figure 2.2.6 - Aquaculture production by volume of main fish groups: percentage of European total aquaculture production in 2014. Source: FAO, 2016.

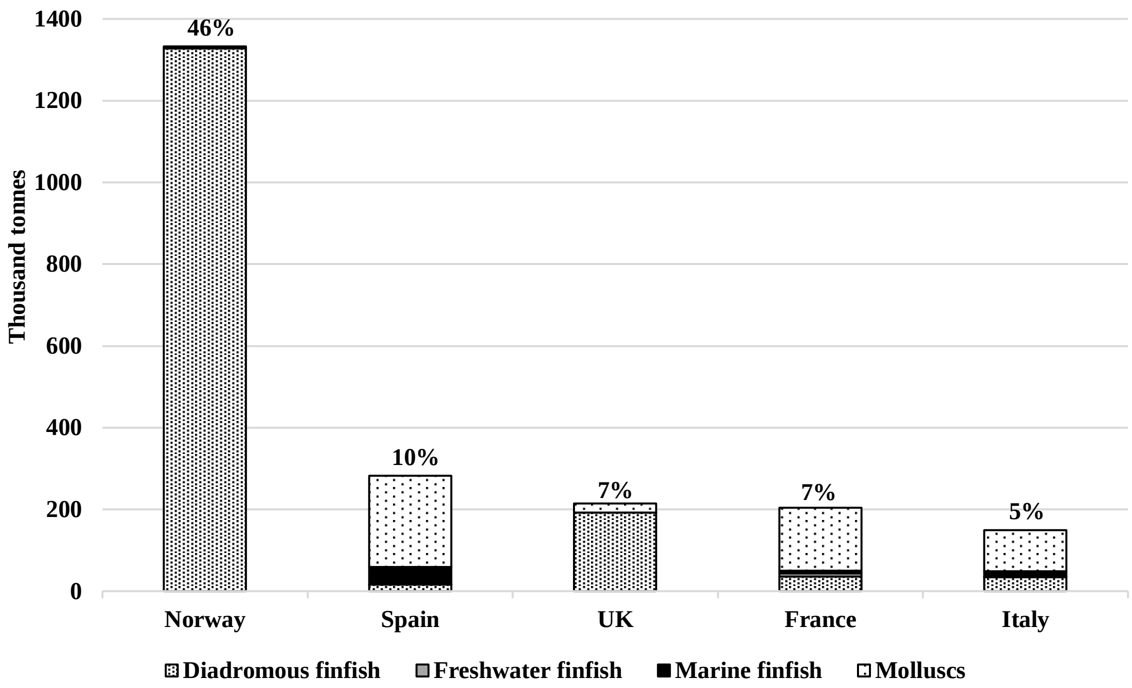

Figure 2.2.7 – Aquaculture fish production of the main producing countries in Europe in terms of volume: percentage of European total aquaculture production in 2014. Values above each column represent the aquaculture production of each country in terms of percentage of the total European aquaculture production in 2014. Source: FAO, 2016.

Atlantic salmon (*Salmo salar*) was the main species reared, especially in Norway, which is the main salmon producer worldwide, accounting for 1,30 million tonnes produced in 2014, followed by the United Kingdom, the third main producer of the species in the world, with a production of 179 thousand tonnes in that year. Rainbow trout (*Oncorhynchus mykiss*) was the second species most reared, with Turkey and Norway as the main producers; European seabass (*Dicentrarchus labrax*) and Gilthead seabream (*Sparus aurata*) fell in third and fourth places, respectively, as main species reared, with Turkey and Greece as the main producers, respectively (EUMOFA, 2016; FEAP, 2015).

In what concerns to molluscs' production, mussels were on the lead of the production, produced especially in Spain, followed by oyster's production, with France as the main producing country (FAO, 2016).

2.3. Aquaculture in Continental Portugal

Portuguese aquaculture focuses mainly on the production of marine fish and mollusc species, which are reared mainly in brackish systems, such as estuaries and coastal lagoons, along the country's mainland coastline; aquaculture in the Portuguese archipelagos is poorly developed and only the Madeira archipelago presents aquaculture production. Rudimental aquaculture methods are thought to have been practised for centuries in Continental Portugal, consisting on imprisoning in salt works and ponds fish and other marine juveniles brought into estuaries and coastal lagoons during high tides, letting them grow to be harvested when at a desirable size for consumption. Aquaculture in Portugal remained essentially a familiar activity until around the end of the 19th century and the beginning of the 20th century, when it started to be regarded as a potentially commercial activity, which could contribute to the country's economy. Rainbow trout (*Oncorhynchus mykiss*) and freshwater species were the first to be farmed at a commercial level, but aquaculture in the country soon evolved to the rearing of mainly marine species.

Despite the mentioned initial advances in aquaculture in Portugal, aquaculture development experienced a slow development over the years, as fishing has always played an important role in the tradition and economy of the country (Ramalho & Dinis, 2011).

Before the 1970's, 80% of fish aquaculture production in Continental Portugal was of mullets; then, during the 1980's, the production was mostly of freshwater rainbow trout and bivalves in intertidal zones. Aquaculture of marine species did not experience true development until the 1990 decade, focusing at first on the rearing of gilthead seabream (*Sparus aurata*) and European seabass (*Dicentarchus labrax*), and more recently on the rearing of turbot (*Scophthalmus maximus*) and sole (*Solea* spp.) (DGRM, 2015).

The greater development of aquacultures in Mainland Portugal took place after Portugal joined the former European Economic Commission, now European Union (EU), in 1986. Being aquaculture a key component of the EU's Blue Growth Agenda, community funds were attributed to Portugal as a member-state for the development of the activity and the Portuguese Government itself invested in aquaculture development as well. The financial support given was intended for the development of research programmes which could enhance knowledge and know-how about aquatic animals' rearing, the construction of new and more suitable establishments, professional training of farmers and financial support to professional organizations (Ramalho & Dinis, 2011). Although Portuguese aquaculture experienced some development in the 1980's, the production decreased significantly in the 1990's, mainly due to a lack of criteria for the application of the available funds and issues on the production methods applied, leading to the unviability of many aquaculture units (Gonçalves, 2013). Nonetheless, despite some fluctuations in aquaculture production over the years, there has been a general rising trend in aquaculture production in Portugal

Comparing to other European countries of the EU, Portugal presents a much slower progress in aquaculture production and development, occupying the 16th place in the rank of total aquaculture production amongst EU member-states in 2014, contributing with less than 1% for the total aquaculture production in terms of volume in the EU (EUMOFA, 2016).

The total aquaculture production in Portugal in 2014 was about 11.3 thousand tonnes (about 0.02% of the world's total aquaculture production), with seafood production accounting for about 10.8 thousand tonnes, around 0.04% of the total aquaculture seafood production in the world (FAO, 2016).

The present work aimed to give an insight on the evolution of the farming of marine species in brackish water, especially in transitional waters' systems, such as estuaries and coastal lagoons, in Continental Portugal over the period between 1999 and 2014.

Production in coastal systems

In Continental Portugal, most of fish farming activities are carried out in estuaries and coastal lagoons, essentially in extensive and semi-intensive systems. Although there is potential for the rearing of freshwater organisms, aquaculture activity in Portugal has focused essentially on the production of marine species since recent developments of aquaculture in the 20th century. The species reared, although marine species, frequently enter the estuaries and coastal systems at some point during their development, and are, thus, well-adapted to the environmental conditions. This fact, along with the availability of juveniles for the grow-out and the high market value of such species, make them the first choices for aquaculture production in Portugal (Ramalho & Dinis, 2011).

Aquaculture production in marine and brackish waters surpassed freshwater production in 1996 and has since then been the main contributor to the total aquaculture production in the country (Figure 2.3.1) representing, in 2014, 92.7% of the total aquaculture production in the country (Figure 2.3.2). In that year, the aquaculture production in marine and brackish waters in Continental Portugal reached its peak in production for the time frame issued, accounting for about 10 thousand tonnes produced (Figure 2.3.1).

Offshore farming is still poorly developed in Portugal but there are already some facilities using offshore cages and longlines for bivalve rearing in the South coast of Algarve that operate since 2008 (DGRM, 2015).

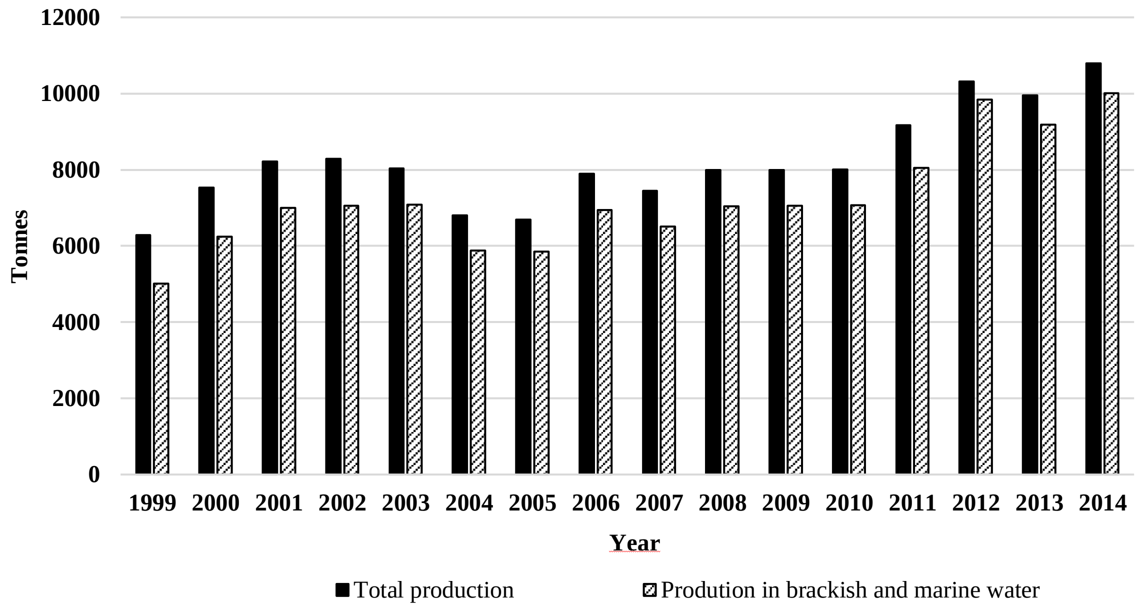

Figure 2.3.1 – Aquaculture production in brackish and marine waters and total aquaculture production over the period 1999-2014, in Continental Portugal. Source: INE, 2000; INE, 2002-2016.

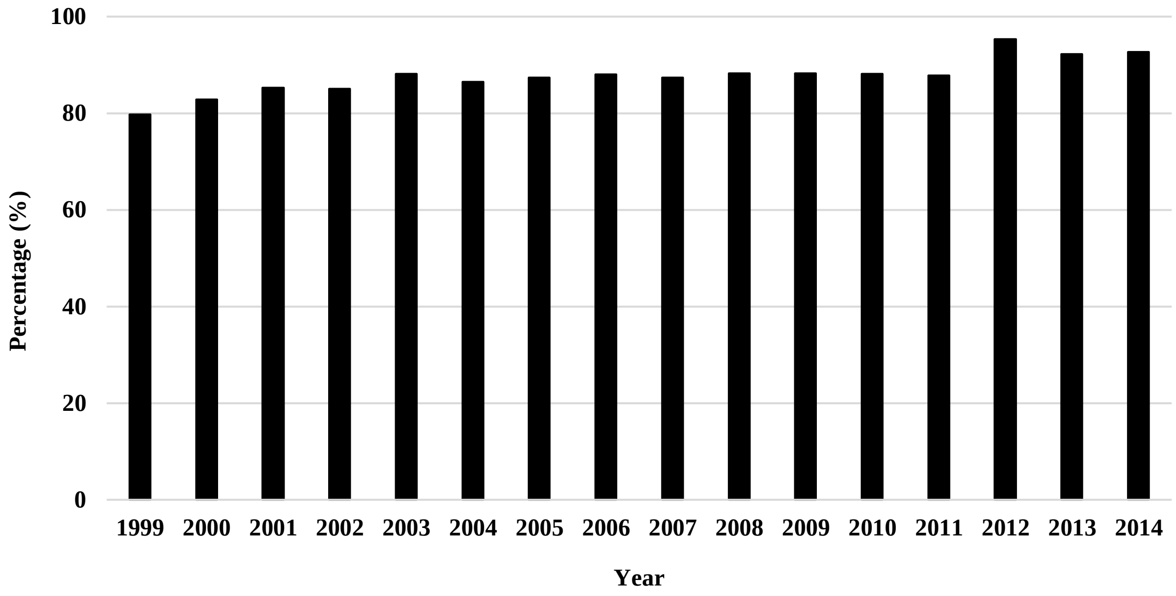

Figure 2.3.2 – Percentage of aquaculture production in brackish and marine waters of the total aquaculture production over the period 1999-2014 in Continental Portugal. Source: INE, 2000; INE, 2002-2016.

Production areas

Aquaculture of marine organisms in transitional waters is mainly carried out in the country's continental land, as mentioned before; only the Madeira archipelago has presented aquaculture production during the period issued in the present work, however, that production was carried out in cages using marine water, which was not the focus of the present work. Thus, the development of aquaculture in Portugal was only assessed in Mainland Portugal, where it is essentially carried out in estuaries and wetlands along the coast, mainly in extensive and semi-intensive production systems especially in the Centre and South of the country. Although offshore farms are beginning to be implemented, there are still very few establishments operating and the production from these facilities is yet notably low comparing to the production in brackish waters. There are currently 20 fluctuant facilities operating offshore, all in the South of Portugal (DGRM, 2015)

There is also no differentiation between the production in brackish and marine waters (from offshore and intensive production using marine water) in official publications, and thus the production referred to in the present work as "production in brackish waters" will comprehend the sum of the aquaculture production in both systems.

For administrative purposes, Continental Portugal may be divided into five main regions: North, Centre, Lisbon and Tagus Valley, Alentejo and Algarve; there are eight main areas for aquaculture in estuarine systems in Mainland Portugal, which are represented in Figure 2.3.3.

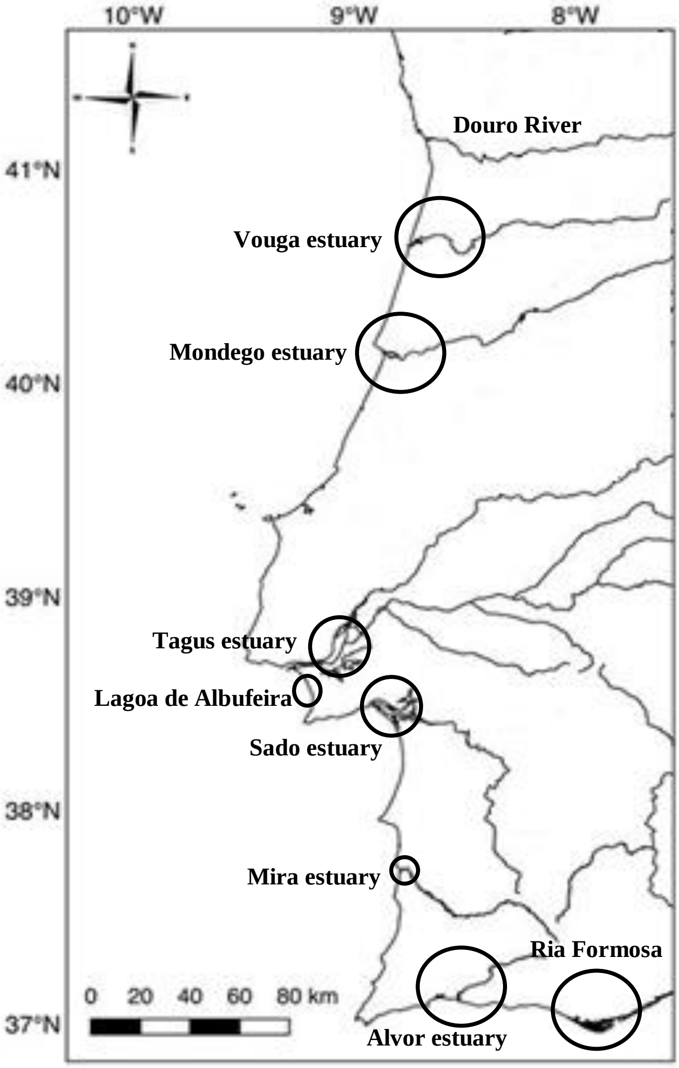

Figure 2.3.3 – Representation of the eight main sites for aquaculture production in Continental Portugal.

One of the most important rivers in Portugal is in the North of the country - the Douro river. However, its estuarine system is of small dimensions and not very suitable for the establishment of aquaculture farms. Thus, freshwater aquaculture is the most representative production in this region, whereas production in brackish and marine waters accounted for a mean of only 4% of the total aquaculture production in the region, during the period between 1999 and 2014. There was a slight increase on aquaculture production in brackish and marine waters in 2002 (about 18% of the total production in the region) but in 2014 it represented only about 0.3% of the production, being the remaining amount of freshwater organisms (INE, 2000, 2002-2016).

At the Centre of Continental Portugal there are two important areas for aquaculture in brackish and marine waters: the Vouga estuary and the Mondego estuary.

The Vouga estuary forms a coastal lagoon with several natural ponds, consisting of an important wetland, known as Ria de Aveiro. The Ria covers about 11ha and is a Natura 2000 network site since 2014, listed as a Special Protection Area site under the Birds Directive. River Vouga runs through farm lands and urbanized areas, such as the city of Aveiro, during its course, being subjected to the input of many pollutants from runoff and domestic wastewater (Van der Weijden & Pacheco, 2006).

The Mondego estuary is, since 2005, a site under the Ramsar Convention on Wetlands. Along its course, the Mondego river runs through highly urbanized areas (such as the city of Coimbra), agricultural fields and industrialized areas, until reaching its 1600ha estuary; the estuary itself is surrounded by urbanized (city of Figueira da Foz) and industrialized areas. The human pressures stated above contribute to the pollution of the river and the estuary, being the main sources of pollution urban wastewater (which is often only partially treated) and chemical products (eg. pesticides, fertilizers) from agricultural water runoff and industrial sewage (Nunes et al., 2011).

Both Ria de Aveiro and Mondego estuary are very suitable for aquaculture in extensive and semi-intensive systems, which are known to be practised for long in these areas, essentially in non-operational salt works. Most of the recent facilities for aquaculture production in this region are, in fact, modified salt works adapted for the production of fish mainly in semi-intensive systems. Aquaculture in estuaries in the Centre region represented a mean of about 97% of the total aquaculture production in the region in the period between 1999 and 2014, accounting, in 2014, for 99.8% of the total aquaculture production in the region. Nonetheless, there are some concerns to be taken into consideration when operating with these rearing methods, as fish farmers need to closely maintain the water quality of their tanks, given that, from time to time, greater amounts of pollutants may reach the estuary and fluctuations in the estuary water quality may potentially affect the production (Nunes et al., 2011; Leitão et al., 2007).

The Lisbon and Tagus Valley area comprises two main areas for aquaculture in brackish waters – the Tagus estuary and Lagoa de Albufeira – and a part of the Sado estuary as well.

The Tagus estuary is the largest estuary in Portugal and one of the largest in Europe, with an area of approximately 32000ha, with about half of that area being a Natural Reserve. The city if Lisbon occupies the estuary's margins, where various activities developing, such as industries, fisheries, an important harbour, resulting in intense maritime traffic and other associated activities (Guerreiro et al., 2015; ICNF, 2017a). Aquaculture in the Tagus estuary focuses mainly on the production of oyster.

The Lagoa de Albufeira is a coastal lagoon with an area of 130ha, with low water renewal and no connection to the ocean after the spring tides, after which a sand barrier is formed. This would isolate the lagoon from water renewal through ocean tides, if it were not for a tidal inlet which is artificially opened to allow the renewal (Coutinho et al., 2012).There is virtually no freshwater aquaculture production in the Lisbon and Tagus Valley area, which makes aquaculture in transitional waters the whole of aquaculture production in the area, apart from the years 1999 – with a production of 23 tonnes of freshwater organisms – and 2002, with a production of 3 tonnes of freshwater organisms (INE, 2000; INE, 2002-2016).

The Sado estuary, comprised between the Lisbon and Tagus Valley and Alentejo regions, is a Natural Reserve since 1980, extending for about 24000ha, being the second largest estuary in Portugal. Most of its area corresponds to the natural reserve (about 23160ha), but the estuary is still subjected to many anthropogenic pressures along its margins and whereabouts, namely several industries (especially on the northern margin), including paper, fertilizers, yeast, food and naval industries, the Setúbal harbour and related activities, the city of Setúbal and copper mines by which the Sado river runs through. Furthermore, the rice fields along the estuary's margins contribute to the input of pollutants and nutrients to the system. Despite all the pressures stated, the Sado estuary is still very suitable for salt and aquaculture production (Caeiro et al., 2005; ICNF, 2017b).

The Alentejo region comprehends as well a small area aimed for aquaculture production (about 105ha), in the Mira estuary, a low impacted system where the main pollution sources are from agriculture fields, tourist-related activities during the summer, and urban waste from the small village of Vila Nova de Mil

Fontes (Costa, Catarino, & Bettencourt, 2001; Vasconcelos et al., 2007). The total aquaculture production in Alentejo is in transitional waters, apart from the year 2006, with the production of 4 tonnes of freshwater products (over a total of 757 tonnes of total production) (INE, 2000; INE, 2002-2016). Although not being an area with great aquaculture production potential, the Alentejo region is one of the few regions in Portugal with natural occurrence of the Portuguese oyster (*Magallana angulata*) (Ramalho & Dinis, 2011).

Algarve comprises two main areas for aquaculture – Alvor estuary and Ria Formosa lagunar system. Ria Formosa is a coastal lagoon extending for about 16300ha, of which about 2000ha are occupied by salt works and aquaculture ponds. This coastal lagoon is a Ramsar Convention on Wetlands and Natura 2000 network site, and receives water input from small watercourses and from tidal exchanges with the Atlantic Ocean (Gamito, 1997). Although there are few industry facilities along the lagoon's margins, they are heavily urbanized and include some areas of intensive agriculture and animal rearing, which are potential sources of pollution and high nutrient input to the basin (raising the risk for eutrophication of the lagoon) (Newton & Icely, 2002). The basin is also profoundly modified from its natural structure, due to coastal engineering, with the construction of artificial inlets in the western part of the lagoon, construction of dykes to retain freshwater from water streams (such as Ribeira de São Lourenço), two sewage treatment plants operating since 2000 and the Faro Airport constructed on the mudlflats (Newton & Icely, 2002).

The Alvor estuary is a Natura 2000 network site since 2006 and many activities are developed on it, such as tourism, fishing and aquaculture production. The margins and adjacent land are quite urbanized and used for animal rearing, which may pose pollution threats to the estuary (Mateus et al., 2016).

All the production in the Algarve is on brackish and marine waters and it is the region that most contributes for aquaculture production in Portugal, accounting for 44% of the total aquaculture production in the country in 2014 and for 48% of the production in Portugal on brackish and marine waters in the same year (INE, 2016).

Establishment types

There are two main types of aquaculture establishments: hatcheries, aimed for the production, by artificial means, of the different development stages of a species, and rearing units, intended for the grow-out of the organisms, from a juvenile stage to the marketable size.

Although there were some hatcheries in the beginning of the century in Portugal, the number of this type of establishment has decreased greatly, being grow-out units the main aquaculture facility type in Portugal, as presented in Figure 2.3.4.

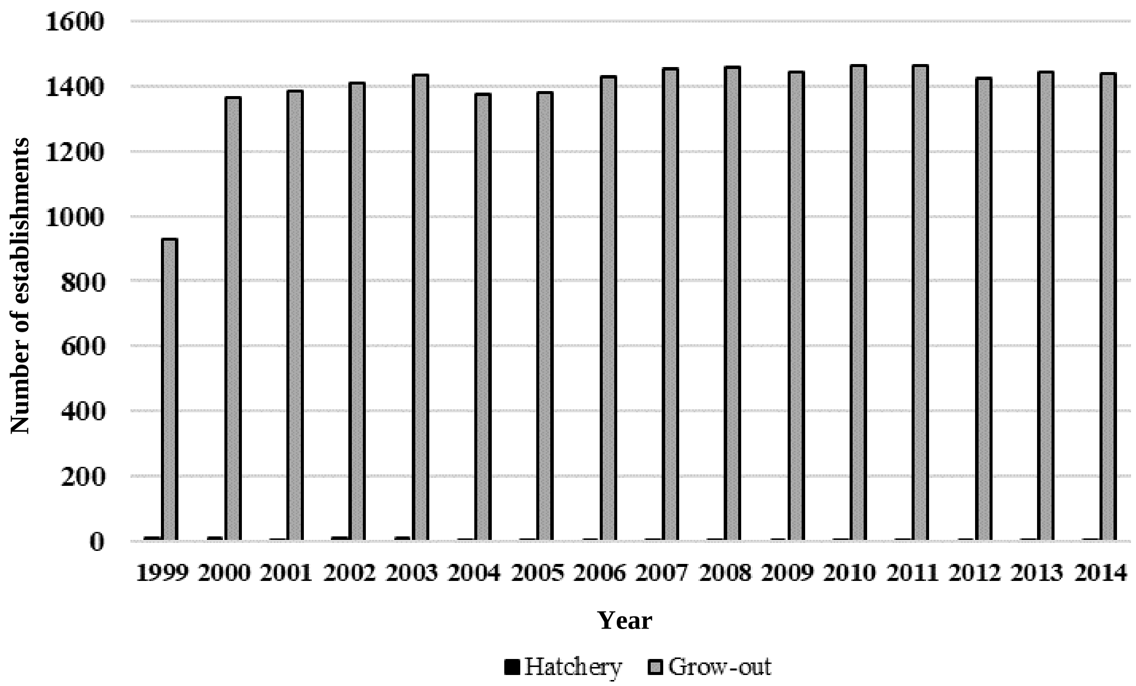

Figure 2.3.4 – Number of hatchery and grow-out units over the period 1999-2014 in Portugal. Source: INE, 2002-2016.

Production systems

The most common aquaculture production systems in Portugal are extensive systems aimed mainly for the rearing of molluscs and semi-intensive systems for the rearing of fish.

For the time frame issued in the present work, extensive aquaculture production has oscillated somewhat over the years but has always maintained high production numbers, consisting mainly on the rearing of molluscs. Production in semi-intensive and intensive systems, specially aimed for the production of fish, have suffered noticeable changes over the years: the break in the production in semi-intensive systems, between 2008 and 2011, was probably due to the conversion of facilities for the rearing of fish using semi-intensive systems in facilities aimed for mollusc production in extensive systems (INE 2000; INE 2002-2016; DGRM, 2017). The contribution of each aquaculture rearing system to the total aquaculture production in Continental Portugal is shown in Figure 2.3.5.

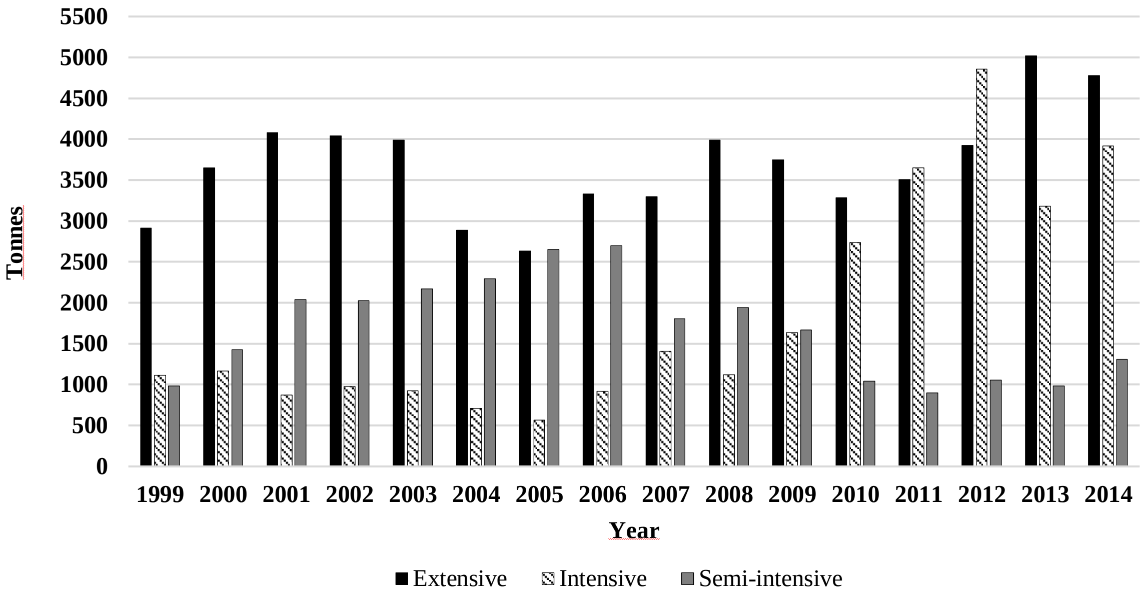

Figure 2.3.5 – Aquaculture production in each production system in brackish and marine waters in the period from 1999 to 2014. Source: INE 2000; INE 2002-2016.

In 2011, production in intensive systems surpassed for the first time (during the time frame issued) the production in extensive systems due to the production of turbot. This finfish is reared essentially in intensive systems and in 2011 its production was of 3197 tonnes alone, contributing to a production of 3648 tonnes in intensive systems, while the total production in extensive systems (including fish and molluscs), was of 3504 tonnes.

In 2013 the production of turbot declined from 4406 to 2353 tonnes, causing the decrease of intensive production, at the same time that mussels and oyster production grew significantly, enabling a new peak of production in extensive systems.

The number of establishments using each rearing system is represented in Figure 2.3.6. Although the number of establishments using intensive systems of production is much smaller than the ones using extensive systems, when comparing the production numbers from each system, it is clear that the capacity of production is greater in intensive systems.

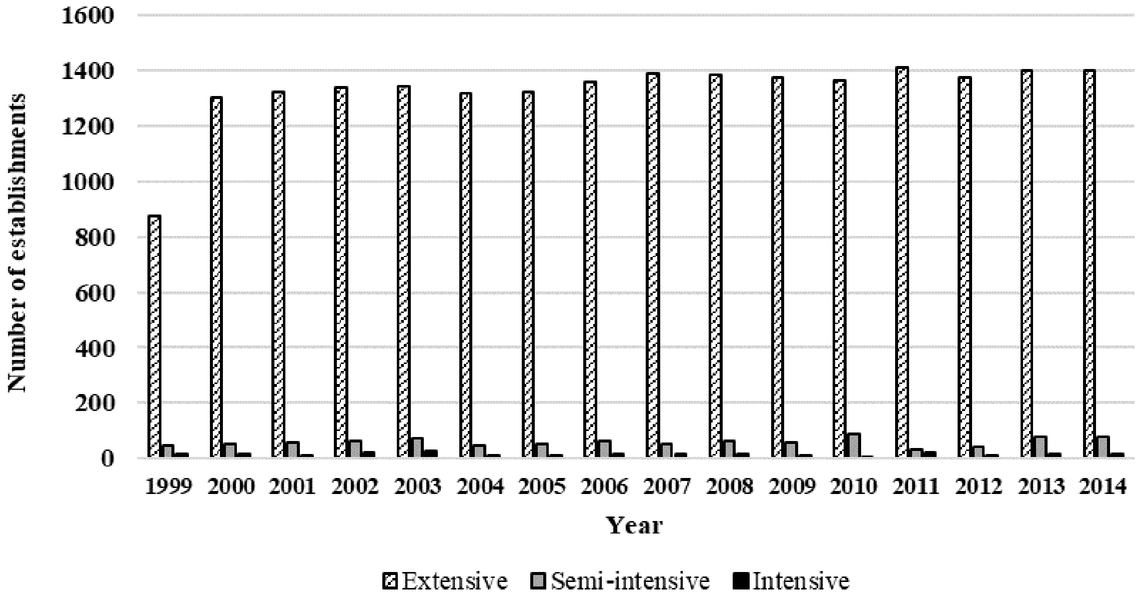

Figure 2.3.6 – Number of establishments using extensive, semi-intensive or intensive production systems in Portugal between the period from 1999 to 2014. Source: INE 2000; INE 2002-2016.

Main cultured species

The aquaculture production in Portugal focuses essentially on the rearing of marine fish species, accounting for about 48% of the total national aquaculture production, and mollusc production, which accounted for about 45% of total national aquaculture production in 2014 (INE, 2016, EA, 2017). Crustaceans are farmed in low quantities and have little expression in the country's aquaculture production and thus their contribution to aquaculture production in Portugal is included in the percentage of molluscs' production. Turbot, clams, mussels, oysters, gilthead seabream, trout and European seabass were, by that order, the seven main species cultured in Portugal in 2014; only trout is reared in freshwater systems (INE, 2016). The comparison between fish and mollusc production in marine and transitional waters in Portugal over the years is presented in Figure 2.3.7.

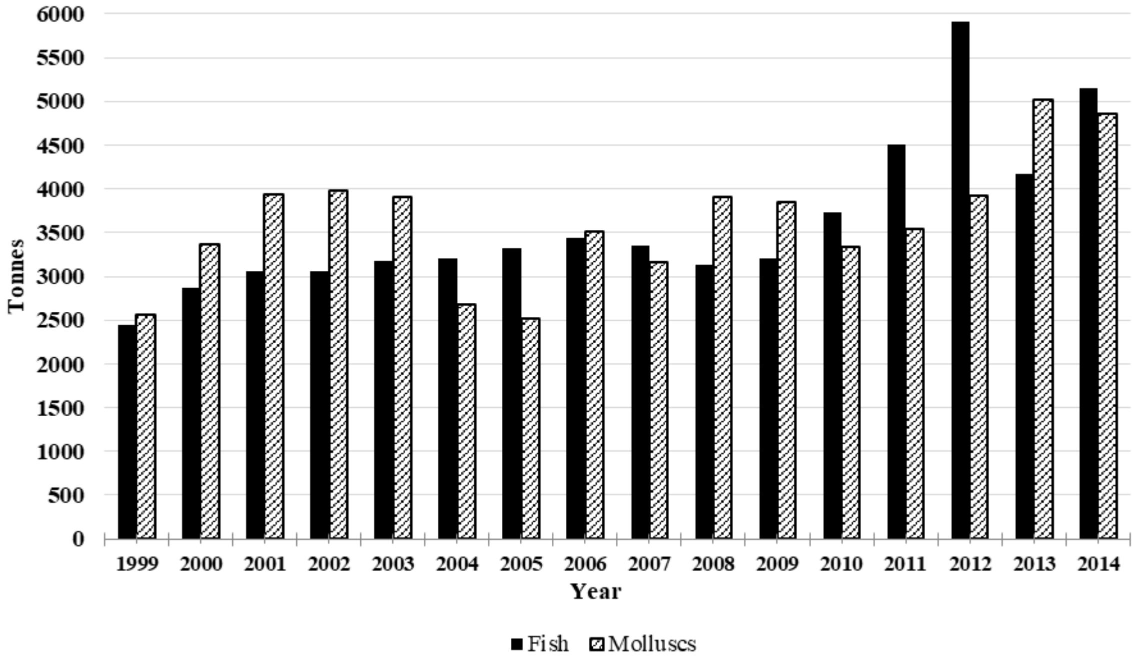

Figure 2.3.7 – Fish and molluscs aquaculture production in brackish and marine waters in Portugal, over the period from 1999 to 2014. Source: INE 2000; INE 2002-2016.

Fish production

According to the most recent data, fish production in brackish and marine waters in 2014 was about 51% of the total aquaculture production in those water systems in Continental Portugal (INE, 2016); the main farmed species in that year was turbot, accounting for about 70% of the total fish production, followed by gilthead seabream, representing about 21% of the total production and European seabass was the third most cultured marine fish species that year, accounting for 8% of fish production. Other species were farmed, although in far lower amounts, such as the sole, with a production of 83.4 tonnes, corresponding to 2% of the total fish production and meagre (*Argyrosomus regius*), with 5 tonnes produced, about 0.1% of total production in 2014 (INE, 2016).

Although turbot has been the most cultured marine fish species in Portugal since 2010, it is essentially reared in intensive systems using water obtained directly from the ocean and not from transitional waters, which is not the focus of the present work; therefore, turbot production will not be further discussed. The production numbers of gilthead seabream and European seabass, which are the two main fish species reared in transitional waters in Continental Portugal, are represented in Figure 2.3.8.

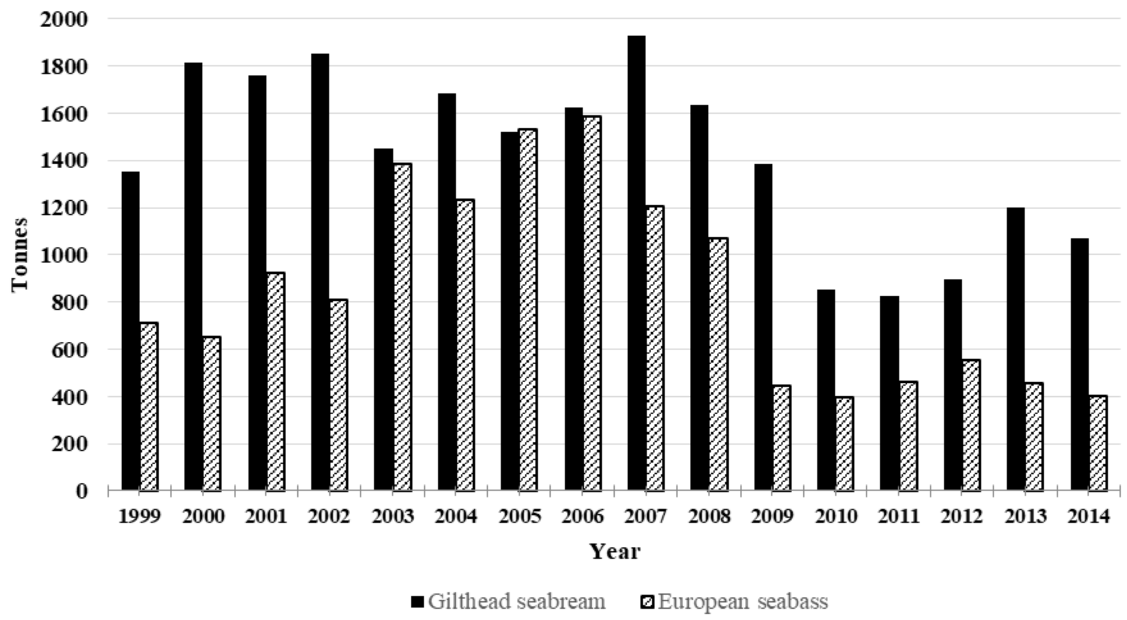

Figure 2.3.8 – Total aquaculture production of the two main marine fish species cultured in brackish and marine waters in Continental Portugal during the period from 1999 to 2014. Source: INE 2000; INE 2002-2016.

There was no significant increase in aquaculture fish production between 2000 and 2008, essentially due to a big output of fish from Greece and Turkey to the European market; this meant a drop in fish price in the European market, with which Portuguese fish farmers could not compete, due to the cost of production that often surpassed the selling income (Ramalho & Dinis, 2011).

Extensive production of fish is practised essentially in inactive salt works, usually in polycultures. This way of production probably began when the salt market started to struggle with some difficulties (Ramalho & Dinis, 2011). However, this system of production is not as productive for fish as semi-intensive or intensive systems, due to the fact that only very low densities of individuals can be achieved. This may be on the origin of the decaying of this practice for the rearing of fish over the years, representing nowadays, according to the most recent data, only about 1% of the fish production in brackish and marine waters. Gilthead seabream and European seabass production values in extensive systems are represented in figure 2.3.9.

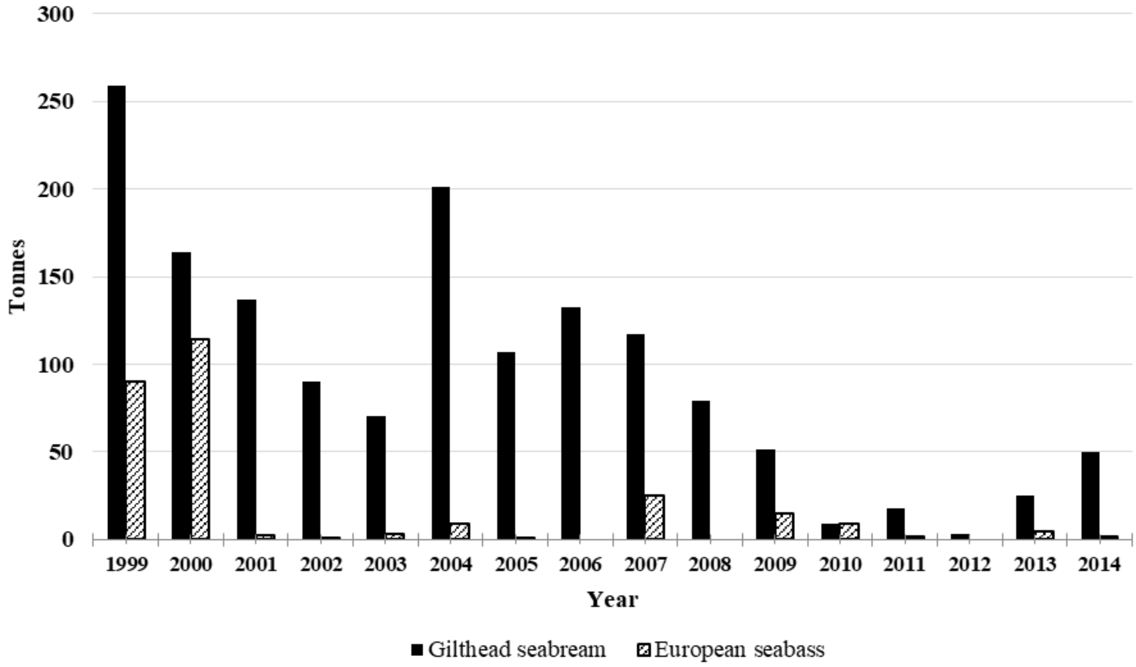

Figure 2.3.9 – Aquaculture production of gilthead seabream and European seabass in extensive systems in Continental Portugal, during the period from 1999 to 2014. Source: INE 2000; INE 2002-2016.

Semi-intensive fish cultures are directed mainly to the rearing of gilthead seabream and European seabass, especially in the Centre and South of Continental Portugal. The rearing cycles differ according to the geographical site: water temperature is generally lower in the central regions when compared to southern farms, with harvesting cycles taking longer in the Centre of Continental Portugal, being of approximately 24 to 26 months (from the juvenile stage, about 10g, to the marketable size), whereas, in the southern regions, cycles last for about 14 to 20 months (Ramalho & Dinis, 2011).

In semi-intensive systems, the quality of the estuarine water used is one of the most pressing parameters to be taken into account by the farmer, as well as the nutrient control, given that fish do not feed only in the food given by the farmer, but also on organisms and other organic matter brought in by the river flow and the ocean tides. Therefore, these parameters must be carefully analysed to avoid the loss of high densities of fish. Predation by birds and some mammals is also to be taken into account and rearing sites may be protected against the action of such animals (Ramalho & Dinis, 2011).

The volume of production of gilthead seabream and European seabass from 1999 to 2014 in semi-intensive systems is shown in Figure 2.3.10.

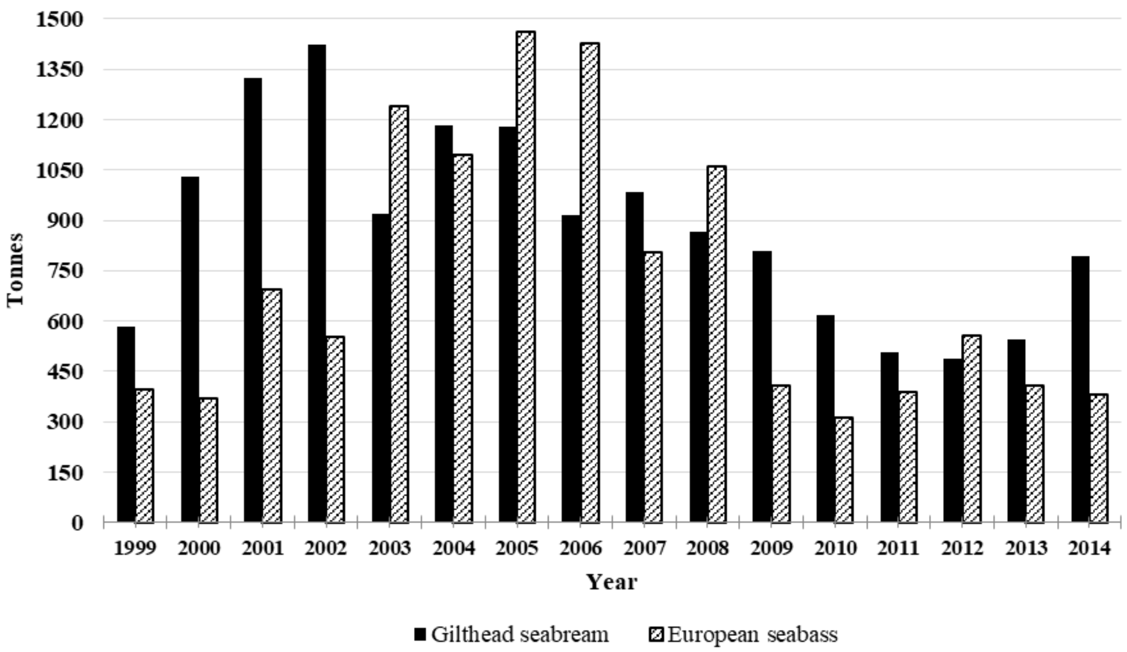

Figure 2.3.10 – Aquaculture production of the two main cultured fish species in semi-intensive systems in Continental Portugal, during the period from 1999 to 2014. Source: INE 2000; INE 2002-2016.

Gilthead seabream was the most cultured marine fish species in Continental Portugal until 2010, when the production of turbot peaked. This species is many times reared in polycultures, especially with seabass, which has proven to be very effective (Dinis et al, 1999). Since 2010, it has been the second most cultured species in the country.

European seabass is one of the most appreciated fish in Portugal and is essentially cultured in semi-intensive systems, usually in polycultures with gilthead seabream, as mentioned above, being the third most cultured fish species in Portugal.

Mollusc production

Bivalve molluscs represent 45% of the total aquaculture production in Portugal. The most produced species is the clam (*Ruditapes decussatus*), accounting for about 50% of the total mollusc production, followed by mussels (*Mytilus edulis*) and the combined production of Portuguese oyster and Pacific cupped oyster (*Magallana gigas*). The production of the mentioned species in the time frame issued is represented in figure 2.3.11.

The production of molluscs in Continental Portugal is mainly carried out in extensive systems, although there is mollusc production in semi-intensive systems as well, mainly of oysters. Extensive rearing of molluscs is essentially carried out in parks, in their natural substrate (mud or sand) (Ramalho & Dinis, 2011), or in ropes (mainly for mussels production).

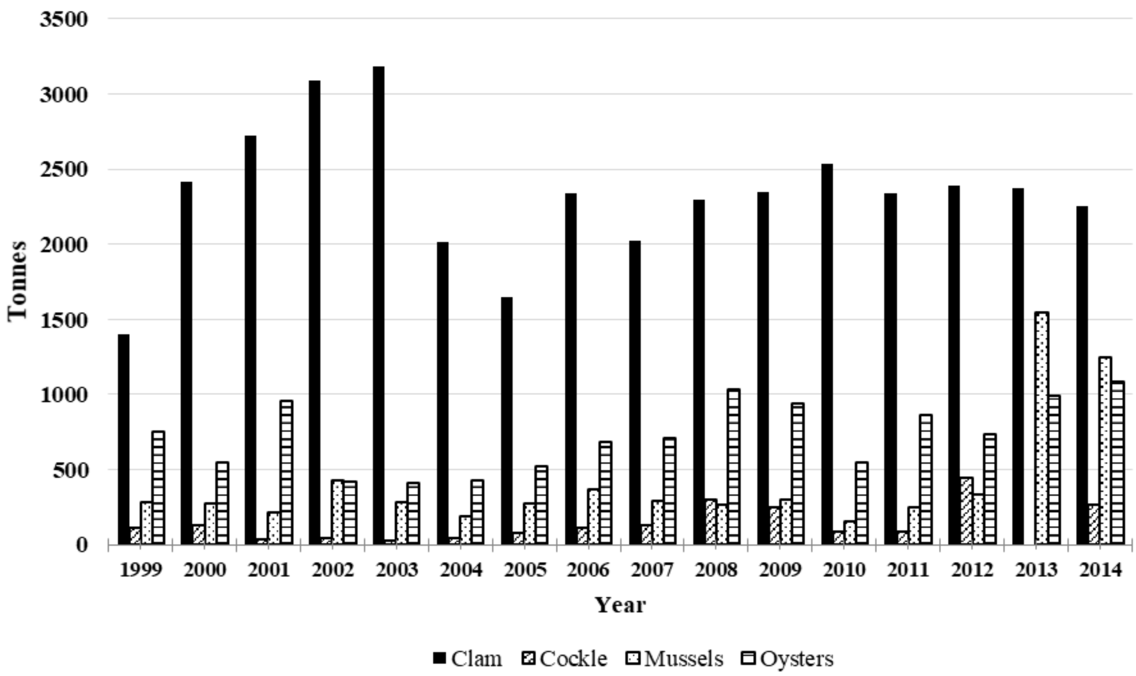

Figure 2.3.11 – Total aquaculture production in transitional waters of the four main cultured mollusc species in Portugal during the period from 1999 to 2014. Source: INE 2000; INE 2002-2016.

The production of molluscs is essentially extensive and only mussels were produced in intensive systems, in the years 2009 and 2011, with a production of 30 and 62.5 tonnes, respectively (INE 2000; INE 2002-2016).

The production of molluscs in semi-intensive systems occurred occasionally over the time frame issued but in quite low quantities. The most expressive production in semi-intensive systems was the production of Pacific cupped oyster in 2006 with 250 tonnes produced, and the production of 125 tonnes in 2014; clams also registered a considerable production of 88.8 tonnes in 2006 and 63.4 tonnes in 2010, still, for the remaining time, the production in semi-intensive systems was nearly imperceptible (INE 2000; INE 2002-2016).

Ruditapes decussatus, the main clams species reared in Portugal, is the most produced species of molluscs in brackish and marine waters and the second most produced species in Portugal. Clams are reared in parks in intertidal zones (Ramalho & Dinis, 2011), in extensive systems, essentially in the south of Portugal. Clam production grew continuously from 1999 to 2003, with the highest growth registered occurring between 1999 and 2000, where a production of around 1404 tonnes in 1999 rose to a production of 2417 tonnes in the following year. The peak of production of these organisms was in 2003, with 3186 tonnes produced, but then dropped to 2014 tonnes in 2004 and to 1647 tonnes in 2005, almost matching the production values of the year 1999. The clam production has, since then, remained rather regular, with a rough average of a little more than 2300 tonnes produced each year. Nonetheless, it represented 46% of the total production of molluscs and about 23% of the total aquaculture production (fish and molluscs) in brackish and marine waters in Continental Portugal in 2014 (INE 2000; INE 2002-2016).

The production of mussel remained quite discrete from 1999 to 2012, revealing a peak from 338 tonnes in 2012 to 1.5 thousand tonnes in 2013. Although mussels' production dropped somewhat the following year, with a production of 1.2 thousand tonnes in 2014, it is the second most produced mollusc in Portugal.

Although most of the aquaculture of mussels in Continental Portugal uses long lines, in extensive systems, there was some production in intensive systems in 2009 (30 tonnes) and in 2011 (62.5 tonnes).

By the end of the 19th century, the Portuguese oyster *Magallana angulata* was the most appreciated delicacy demanded by the European market (especially France), which led to an increase in the capture in the Tagus estuary during that period, reaching, during the 1930's, an exportation of 13 thousand tonnes per year (Ramalho & Dinis, 2011). It was the only oyster species being reared in Continental Portugal until 2004, when its production dropped abruptly and was replaced by the production of the Pacific oyster. The Portuguese oyster production restarted in 2009 and has been growing ever since (INE 2000; INE 2002-2016).

Oysters are usually reared in net bags placed on the substrate, which may subject them to predation. The culture in long-lines and floating devices, used mainly in Algarve, protects animals against non-swimming predators, as the organisms are suspended in the water column (Ramalho & Dinis, 2011).

Cockle (*Cerastoderma edule*) production has fluctuated during the time frame issued, maintaining a low production between 2001 and 2005, rising in 2006 and reaching a production of about 300 tonnes in 2008, the second biggest production of cockle in the time period covered by the present work. After two years (2010 and 2011) with a production of roughly 90 tonnes each year, in 2012 the greatest production of cockled was reached, accounting for 449.2 tonnes produced. However, there was no production of this species in 2013, recovering somewhat in 2014, with a production of 254 tonnes. The cockle production is carried out essentially in parks, using extensive systems (INE 2000; INE 2002-2016; Ramalho & Dinis, 2011).

2.4. Final Remarks

As one of the leading fish consumers *per capita* in the world, it would be expected that Portugal had already developed aquaculture practices as a means of matching the market demand and complement capture fisheries, but production is still far from matching such demand, and imports cannot be avoided.

Most of the aquaculture facilities in Portugal focus on the production of marine finfish and bivalve molluscs, essentially carried out in estuaries and coastal lagoons, in extensive and semi-intensive systems. Although facilities using intensive production systems are more efficient in the rearing of organisms and could attain greater production numbers, they are only used for a small number of species, essentially fish.

In Continental Portugal, aquaculture production in coastal systems reached a total of 10.8 thousand tonnes in 2014, the greatest production for the time frame issued – from 1999 to 2014 – representing about 92.7% of the country's total aquaculture production.

Offshore farming in Portugal is in development but, for the present time, it resumes to few cages and longline systems for bivalve molluscs production in the South coast of Algarve, operating since 2008.

Fish production accounts for about 51% of the total aquaculture in coastal systems in Continental Portugal, and focuses on the production of gilthead seabream and European seabass, which accounted for 21% and 8%, respectively, of the total fish production in 2014. Most fish production is carried out in semi-intensive systems in the Centre and South of Portugal.

Mollusc production represented about 45% of the aquaculture production in 2014, focusing on the rearing of clam – about 50% of the total mollusc production –, followed by the production of mussels and oysters; the rearing of this group is carried out essentially in extensive systems.

Although a small producer country, ranking 20th in terms of aquaculture volume production in Europe, aquaculture production in Portugal showed a rising trend over the years, and research about aquaculture's methodologies and best practises has been developed. It is a sector that is given greater importance to every year and should continue to expand and develop throughout the following years.

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Chapter 3

Characterization of the nutritional value of farmed European seabass and gilthead seabream from semi-intensive aquaculture in two estuaries in Portugal

3.1. Abstract

The nutritional value of cultured fish was assessed for European seabass and gilthead seabream specimens reared in semi-intensive systems in two Portuguese estuarine systems. Quantification of total protein, carbohydrate and fatty acid profiles was carried out to test the differences between the organoleptic composition of organisms of the same species reared in four different aquacultures. No significant differences were found among groups of both species regarding protein content. A significant influence of the rearing site was found for European seabass regarding saturated, monounsaturated and highly unsaturated fatty acid (SFA, MUFA and HUFA, respectively) contents, either between estuaries and within each estuary; in gilthead seabream, SFA, MUFA, polyunsaturated fatty acid (PUFA) and HUFA contents were also influenced by the rearing site. Eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (AA) and linoleic acid (LA) contents were dependent on the fish rearing site as well and, in general, seabass and seabream fatty acid content was higher in organisms reared in the Sado estuary, when compared to the reared in the Mondego estuary. Carbohydrate analysis showed a significant influence of the rearing site on free sugars and polysaccharides content in fish of both species. The present study supported the existing evidence that semi-intensive rearing systems are subjected to the variability of extrinsic factors in the rearing sites, which may influence the final nutritional value of the same species produced in different facilities. From a consumer's perspective, such differences may come as a disadvantage for this rearing method, as it is expected for a product to provide equal nutritional properties and benefits regardless its origin, especially within the same country.

Key-words

Semi-intensive aquaculture, estuary, *Dicentrarchus labrax*, *Sparus aurata*, nutritive content

3.2. Introduction

Aquaculture has rapidly developed over the past few decades, already accounting for more than half of the seafood supply for human consumption worldwide. Fish aquaculture represents 68% of the total global aquaculture production (FAO, 2016).

Fish are considered healthy food, due to their high content in polyunsaturated fatty acids (PUFA) and highly unsaturated fatty acids (HUFA), especially the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (EFSA, 2014; Tocher, 2003), high-quality protein composition and other essential nutrients, such as vitamins and minerals (Kromhout et al., 2016; EFSA, 2014; Webster-Gandy, Madden, & Holdsworth, 2006). Nonetheless, fish development depends on their intrinsic characteristics and the extrinsic environmental conditions they are subjected to, reflecting on their organoleptic and, consequently, nutritional compositions (Alasalvar et al., 2002; Borresen, 1992; CONTAM, 2005; Cordier, Weber, & Zwingelstein, 2002; Fuentes et al., 2010; Grigorakis, 2007; Huss, 1995; Izquierdo et al., 2003; Lal, 1989).

The final nutritional composition of aquaculture products depends greatly on the culture system used (Flos et al., 2002), which range from none or little control over the extrinsic factors to which the products are subjected to in extensive systems, as water and feed provided to the organisms are from the natural source, to total regulation of extrinsic rearing conditions in intensive systems, with a high control over the desired organoleptic composition of the final product.

Semi-intensive systems' characteristics lay in the middle of the latter two. The water system in which the aquaculture facilities are situated functions as natural water source for the rearing ponds, meaning there is low control of its composition and quality, and organisms are fed by the farmer, controlling most of the fish nutrient intake. However, with every water renewal, organic matter and other organisms eventually enter the rearing ponds and the farmed fish may feed on them as well, possibly influencing their final composition.

As different water sources usually differ in their intrinsic characteristics, as chemical properties, dissolved nutrients and biological communities, and semi-intensive aquaculture systems are poorly controlled, the same species reared in semi-intensive farms in different water systems will most likely present different organoleptic compositions, which may not be desirable from a commercial point of view (Gökçe et al., 2004; Russo, 2009). Moreover, the dimensions of the tanks and the number of individuals per tank may also cause stress to the organisms, and the combined influence of all the stated factors (water quality, environmental parameters, stress in tanks, among others) potentiates differences in the nutritional content of cultured species.

Semi-intensive aquaculture is currently the most common method for fish aquaculture in Portugal and focuses on the rearing of high commercial value marine species in estuarine systems, usually in inactive salt pans modified for fish rearing in semi-intensive regimes. As estuaries are commonly used by marine species as nursery areas and for protection during early stages of development (Costanza et al., 1997; Elliott et al., 2007; McLusky & Elliott, 2004; Peterson, 2003) those species are well-adapted to the estuarine conditions and are thus well suited for rearing in such areas.

The European seabass *Dicentrarchus labrax* (Linnaeus, 1758) and the gilthead seabream *Sparus aurata* (Linnaeus, 1758) are two of the main fish species reared in Portugal in such conditions.
These two fish species are greatly appreciated by consumers, presenting a high commercial interest (Alasalvar et al., 2002; Fuentes et al., 2010; Kyrana & Lougovois, 2015), and are common along the country's coast and well-adapted to the estuarine environment, as they frequently use estuaries as nursery areas.

The Mondego and Sado estuaries are two important estuaries in Portugal for fish rearing, especially for the production of the above-mentioned species.

The Mondego estuary is an intertidal estuary that extends for about 9 km^2, known to be an important nursery area for European seabass, gilthead seabream and other marine species (Leitão et al., 2007; Martinho et al., 2007). The estuary comprises two arms with quite different characteristics and most aquaculture facilities are placed in the southern arm. Amongst non-point pollution sources of the Mondego estuary are the agricultural fields through which the river runs along its course, constituting serious sources of fertilizers and pesticides into the water course, and the partially untreated domestic sewage and industrial waste input to the river, once the river basin supports many populations (Ferreira, et al., 2004; Nunes et al., 2011). The main on-point pollution sources of the Mondego southern arm are the industrial activities in the area, urban waste from the populations nearby, livestock production and aquaculture production itself (APA, 2016b; Martinho et al., 2007).

The Sado estuary is the second largest estuary in Portugal, with an area of approximately 240km^2. The estuarine area is disputed between industrial plants, agricultural fields, aquaculture facilities and natural reserves (APA, 2016a). The northern margin of the estuary comprises most of the industrial facilities working in the area, which are rather polluting, namely the paper, pulp, pesticides and food production facilities (Catarino, Peneda, & Santana, 1987; Lopes da Cunha, 1994). The estuary also comprises a harbour, whose associated activities are great sources of pollution to the water body The city of Setúbal develops along the margins of the estuarine system, acting as a source of pollution essentially via urban waste. The referred working industries, urban waste, agricultural fields, rearing of terrestrial animals and aquaculture production itself are the main on-point anthropogenic pressures jeopardising the water quality of the estuary. (APA, 2016a).

To assess the potential influence of different environmental conditions in the nutritional profile of *D. labrax* and *S. aurata* reared in four aquacultures from the two distinct estuaries, a set of biochemical analysis were conducted, namely the quantification of the total protein and ash content and determination of the fatty acids and carbohydrate profiles of the fish species. These analyses also allowed to relate the nutritional composition of fish species with the commercial diet and feeding methods applied in the aquacultures.

Lipids and their constituents fatty acids act as first source of metabolic energy for fish, allocated for growth, movement (including migrations) and reproduction (Marshall et al., 1999; Tocher, 2003), sometimes exceeding the protein content. In terms of human metabolism, fish are specially important sources of omega-3 HUFAs, which have been proven to be related with the prevention of cardiovascular and autoimune diseases (Harris, Kris-Etherton, & Harris, 2008; Harris, Poston, & Haddock, 2007; Lee et al., 2009; Russo, 2009; Thies et al., 2003), human brain and ocular development since early stages (Clandinin, 1999; Dyall & Titus, 2008; Simopoulos, 2009; Salem et al., 1996), reducing the production of reactive oxygen species (ROS), thus having an antioxidant function (IOM, 2011), among other benefits.

The proteins of fish have a primary structural function as constituents of skeletal muscle and a metabolic role of substrate for cell respiration (Wootton, 1990). Fish is a high protein content food, as referred before, generally with optimal essential amino acid composition for human needs, making them high-quality protein sources (EFSA, 2012).

Carbohydrate content of fish is much less significant than the fatty acid and protein content (Wootton, 1990), but they play a secondary role in energy storage, especially in the form of the polysaccharide glycogen. Regarding human nutrition, fish are not a primary source of carbohydrates intake.

The present study aims to evaluate if the rearing environment of farmed *D. labrax* and *S. aurata*, along with differences in commercial feed brands and feeding methods used, influence differently the development of the species and thus results in different organoleptic compositions of the same species when at a marketable size, influencing the nutritional value of the products, from a consumer's perspective.

3.3. Materials and Methods

Study sites

Fish samples were collected during the summer of 2016, from four different aquaculture farms, two in the Mondego estuary (M1 and M2) and two in the Sado estuary (S1 and S2), as represented in Figure 3.3.1; the four aquacultures produced European seabass and gilthead seabream. Aquacultures M1, S1, S2 used the commercial feed brand Aquasoja standard orange 6 (SORGAL®), whereas aquaculture M2 used a different feed brand (L-6 and D-6 Alterna 3P, Skretting España S.A.); nonetheless, both feeds presented a similar basic macronutrients composition, which is represented in Table 3.3.1. Feeding systems differed between aquacultures: aquacultures M1 as S2 feed the tanks manually, while aquacultures M2 and S1 fed the production tanks with resort to mechanical techniques.

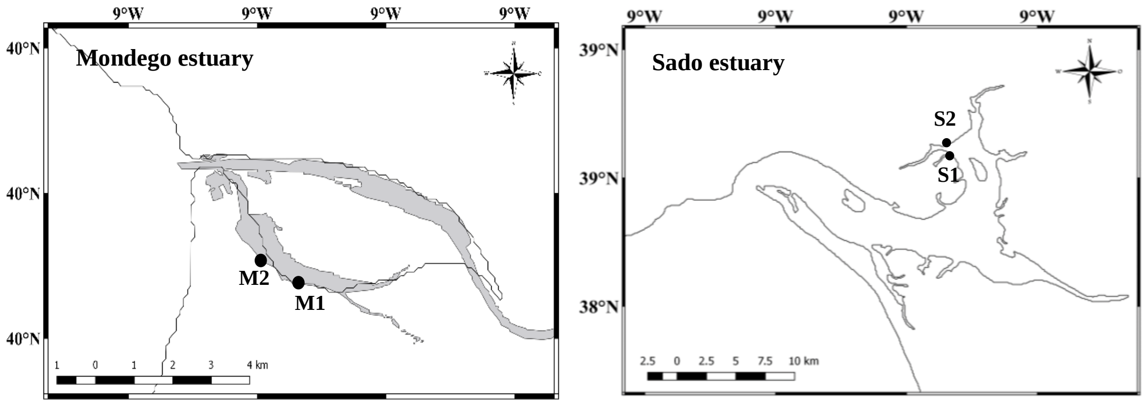

Figure 3.3.1 - Localization of the four aquacultures sampled, two in the Mondego estuary (M1 and M2) and two in the Sado estuary (S1 and S2).

Water characteristics of the different study sites were not directly measured in the present work, having the data been collected from the literature.

Aquacultures in the Mondego estuary are situated in areas subjected to similar pressures, especially industrial effluents, urban waste water, livestock rearing and aquiculture itself; the harbour located in the north arm may also contribute for the pressure on the estuarine systems and the agriculture fields along the estuary's whereabouts also pose some threats in terms of nutrient input, interfering with the water quality (APA, 2016a). In terms of chemical composition of the water, the most recent data available corresponds to 2010 measurements (APA, 2016a), during the summer season, and the following results were obtained: pH = 7.7, salinity = 33.5, dissolved O_2 = 7.90mg/L, ammoniacal nitrogen = 0.1mg/L NH_4, total phosphate content = 0.03mg/L and total nitrate = 0.09mg/L NO_3.

Aquacultures in the Sado estuary are placed in sites subjected to different human pressures: the site in which aquaculture S1 is situated lays closer to the industrial zone of the estuary, thus being more subjected to industrial effluents and pollutants; aquaculture S2, on the other hand, is placed in an area surrounded by agricultural fields and livestock rearing, potentially receiving higher nutrient input from agricultural water runoff. Both sites are significantly influenced by urban waste and, although no recent data on the chemical composition of the water in the Sado estuary was available, the water masses were characterized with good environmental status, according to the European Union Water Framework Directive classification (EU, 2000; APA, 2016b).

Table 3.3.1 – Composition of the diets used in the four studied aquacultures. Values in percentage (%) of total composition.

%	**M1, S1, S2**	**M2**
Crude Protein	42.0	40.5 – 44.0
Crude Fat	17.0	18.0 – 24.0
Crude Ash	11.0	7.2 – 8.3
Crude Fibre	2.0	3.7 – 4.5
Calcium	2.4	1.9 – 2.0
Phosphorous	1.5	1.3 – 1.4
Sodium	0.3	0.4

Sample collection

Market-size cultured European seabass and gilthead seabream adults collected from the four aquacultures were transported in ice and immediately measured for total body weight (balance precision of 0,01g) and total body length (scale precision of 0,1cm), upon arrival at the laboratory (Table 3.2). Fish were then stored at -20ºC until filleting, after which the collected samples of muscle and liver were stored at -80˚C until analysis.

Table 3.3.2 – Total body weight (g) and total body length (cm) measurements of sampled organisms. Values expressed in mean (± SD).

		Aquacultures			
		M1	**M2**	**S1**	**S2**
D. labrax	Weight (g)	308.58 ±19.59	337.30 ± 46.98	345.65 ± 86.80	730.03 ± 54.60
	Size (cm)	30.4 ± 1.2	30.1 ± 1.8	32.3 ± 2.3	39.3 ± 1.7
S. aurata	Weight (g)	337.67 ± 34.41	293.26 ± 22.48	516.20 ± 37.42	556.80 ± 73.33
	Size (cm)	28.3 ± 1.2	26.0 ± 1.1	31.5 ± 1.1	31.5 ± 1.2

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Biochemical analysis

Ash content

Determination of total ash percentage in fish samples was carried out according to the AOAC (2000) procedures. Three replicas per aquaculture of muscle tissue samples, weighting 2.50g, were used to determine ash content of the study groups; samples in crucibles were placed in a muffled furnace at 550ºC overnight and the percentage ash content calculated as indicated in the referred procedure.

Total Protein content

Fillets of fish muscle from each sample were weighted, 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 analysis. Total protein quantification was carried out as described in Bradford, 1976, adapted to a 96-wells microplate; the die used was Protein Assay Dye Reagent Concentrate (Biorad ®), which was diluted in ultra-pure water at a concentration of 1:4. Protein quantification was carried out using a Thermo Multiskan EX Microplate reader.

Fatty acid analysis

Fatty acid extraction from fresh fish tissue and methylation to fatty acid methyl esters (FAMEs) for analysis was carried out as described in Gonçalves et al. (2012), apart from the final vacuum drying, as samples for the present work were stored in liquid form and kept at -80ºC until analysis.

FAMEs identification was carried out through Gas chromatography-Mass spectrometry (GC-MS), using an Agilent Technologies 6890N Network (Santa Clara. CA) equipped with a 0.25 mm internal diameter, 0.1 µm film thickness and 30 m long DB-FFAP column; 1,4 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. An Agilent 5973 Network Mass Selective Detector at 70 eV electron impact mode, scanning the range m/z 40-500 in 1s cycle in full scan mode acquisition was connected to the GC-MS. The initial oven temperature was 80ºC, following a linear temperature increase of 25ºC min^{-1} to 160ºC, after which followed another temperature ramp of 2ºC min-1 to 190ºC and finally an increase of 40ºC min^{-1} until the final temperature of 230ºC was reached and maintained for 5min. Helium was the carrier gas used, at a flow rate of 4.4mL min^{-1} and 2.66 psi of column head pressure. The detector starts operating 4 min after injection, corresponding to solvent delay. The injector ion source and transfer line were maintained ate 220ºC and 280ºC, respectively. Integration of FAME peaks was carried out using the equipment's software; identification of each peak considered the retention time and mass spectrum of each FAME, comparing to the Supelco ® 37 component FAME mix (Sigma-Aldrich, Steinheim, Germany). Quantification of FAMEs was obtained as described in Gonçalves et al. (2012).

Sugar analysis

Sugar extraction from fish tissue began with the recollection of the remaining pellet after centrifugation in the above-mentioned protocol for fatty acid extraction from fresh tissue.

Sugar analysis comprised the quantification of polysaccharides and free sugars (monosaccharides).

The extracts obtained were subjected to different procedures, depending on the type of sugar determination. For polysaccharide analysis, samples were subjected to hydrolysis followed by reduction and acetylation, as described in Coimbra et al. (1996); for free sugar analysis samples were not subjected to hydrolysis but followed the same protocol for reduction and acetylation.

Both polysaccharide and free sugar samples were run through a Perkin-Elmer – Clarus 400 gas chromatography equipment, equipped with a flame ionization detector (GC-FID). A DB-225 (30m length, 0.25mm i.d., 0.15 µm film thickness) GC column was used and oven was programmed to an initial temperature of 200ºC, following a linear temperature increase at 40ºC min^{-1} to 220ºC, this temperature was maintained for 7 min, after which followed another linear increase of 20ºC min^{-1} to the final temperature of 230ºC, maintaining this temperature for 1 min. The carrier gas was Hydrogen, at a flow rate of 1.7 mL min^{-1}. Quantification of sugars was obtained by comparison of the sugar chromatographic peaks to the peaks obtained for the standard used (2-desoxiglucose).

Statistical analysis

Macronutrients profiles of the fish studied were analysed through Principal Component Analysis (PCA), which were carried out to highlight fatty acid composition differences amongst the organisms studied and, thus, the aquacultures. Nested analyses of variance (ANOVA) were conducted to test differences in components among the studied groups.

3.4. Results

Ash content

No significant differences were found in ash percentages among the individuals studied (Table 3.4.1), either between estuaries (for *D. labrax* F=1.441 and for *S. aurata* F=0,128, p-value > 0.05 in both cases) or between aquacultures within the same estuary in both species (for *D. labrax* F=4.126 and for *S. aurata* F=4.271; p-value > 0.05 in both cases).

Table 3.4.1 – Percentage of ash content in the individuals studied, values represented as mean ± SD.

	Aquaculture			
	M1	**M2**	**S1**	**S2**
D. labrax	5.87 ± 3.86	0.80 ± 0.41	2.39 ± 2.10	1.20 ± 0.40
S. aurata	3.73 ± 1.69	1.20 ± 0.41	4.27 ± 2.59	1.33 ± 0.91

Total protein content

Analysis of the protein content between the study groups showed no differences among estuaries (for *D. labrax* F=0.407, for *S. aurata* F=0.802, p-value > 0.05 in both cases) nor between aquacultures within the same estuary (for *D. labrax* F=0.968, for *S. aurata* F=0.571, p-value > 0.05 in both cases), for any of the species (Table 3.5.2). Although differences were not statistically significant, *D. labrax* reared in aquaculture M2 presented the highest mean protein content when compared to conspecifics reared in the remaining aquacultures, corresponding to nearly 53% of the total composition of the fish muscle tissue; the lowest protein content for that species was registered in organisms reared in aquaculture S1. For *S. aurata*, the highest mean protein content was found in organisms reared in aquaculture S2, corresponding to over 63% of the fish muscle composition, while the lowest content corresponded to the organisms reared in aquaculture M2.

Table 3.4.2 – Total protein content (mg/g of fish muscle tissue) of the organisms analysed; values are represented as mean (± SD).

	Aquaculture			
	M1	**M2**	**S1**	**S2**
D. labrax	412.67 (±28.80)	528.82 (±95.63)	409.78 (±153.65)	451.69 (±116.88)
S. aurata	427.68 (±55.59)	407.52 (±242.91)	437.96 (±123.27)	632.97 (±308.53)

Fatty acid content

Analysis of the fatty acid profile of the studied samples showed content in myristic (C14:0), palmitic (C16:0), margaric (C17:0) and stearic (C18:0) acids for saturated fatty acid (SFA) total content, in pentadecenoic acid (C15:1), palmitoleic acid (C16:1), heptadecenoic acid (C17:1) and oleic acid (C18:1) for monounsaturated fatty acid (MUFA) content, PUFA content included linoleic acid (C18:2) and docosadienoic acid (C22:2) and HUFA content comprehended arachidonic (C20:4, AA), eicosapentaenoic (C20:5, EPA) and docosahexaenoic (C22:6, DHA) acids, whose values are represented in Table 3.5.3, for every study group analysed.

Palmitic acid was the most abundant saturated fatty acid found in both species, contributing to over 70% of the total SFA content in *D. labrax* and *S. aurata* reared in aquacultures M1 and M2; the referred fatty acid contribution to the total SFA content was nearly 63% and 72% in *D. labrax* from aquacultures S1 and S2, respectively, and close to 80% and 90% in *S. aurata* reared in aquacultures S1 and S2, respectively.

Oleic acid was, apart from the observed in European seabass from aquaculture S2, the most abundant monoenoic fatty acid, contributing to over 60% of MUFA total content in *S. aurata* in all aquacultures, and close to and over 50% in *D. labrax* from aquacultures M1 and M2, respectively.

Concerning PUFA content, the fatty acid profile was not “typical”, when compared to polyunsaturated fatty acid profiles from other cultures of seabass and seabream, assessed in similar studies, which will be later discussed. Linoleic acid was only found in fish from aquacultures in the Mondego estuary for *D. labrax* specimens, and was the sole PUFA found in European seabass reared in aquaculture S2; LA was found in all *S. aurata* individuals tested, and contributed to 15% to 20% of total PUFA content. Docosadienoic acid was the most abundant PUFA, when present, accounting for 73% of the total PUFA content in *D. labrax* from aquaculture M1 organisms and was the sole PUFA found in *D. labrax* reared in aquaculture S2; the referred fatty acid contribution to the polyunsaturated fatty acid content in *S. aurata* specimens ranged from 80% to 86% in organisms from the four aquacultures.

The omega-3 series DHA was the most abundant fatty acid in both species, contributing to nearly 70% of the total HUFA content in the European seabass reared in aquacultures M1 and S1 and over 80% in *D. labrax* from aquacultures M2 and S2. In *S. aurata*, DHA contribution was even higher, accounting, in general, for nearly 90% of the total HUFA content of the organisms studied.

Table 3.4.3 – Total content (mg/g of fish tissue) of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and highly unsaturated fatty acids (HUFA) in the fish groups analysed; results are expressed in mean values.

	D. labrax				***S. aurata***			
Fatty Acid	**M1**	**M2**	**S1**	**S2**	**M1**	**M2**	**S1**	**S2**
14:0	0.00	0.01	0.01	0.04	0.01	0.01	0.04	0.07
16:0	0.08	0.09	0.27	1.31	0.15	0.08	1.07	1.60
17:0	0.00	0.00	0.06	0.12	0.00	0.00	0.00	0.00
18:0	0.03	0.02	0.09	0.36	0.05	0.02	0.23	0.15
Total SFA	**0.11**	**0.12**	**0.43**	**1.83**	**0.21**	**0.11**	**1.34**	**1.82**
15:1	0.06	0.05	0.10	0.98	0.05	0.03	0.23	0.16
16:1	0.01	0.02	0.05	0.26	0.03	0.02	0.21	0.36
17:1	0.04	0.04	0.05	0.55	0.02	0.02	0.09	0.00
18:1	0.10	0.15	0.13	0.51	0.16	0.11	1.06	1.68
Total MUFA	**0.21**	**0.26**	**0.33**	**2.31**	**0.26**	**0.18**	**1.59**	**2.20**
18:2 (LA)	0.04	0.05	0.00	0.00	0.05	0.05	0.56	0.71
22:2	0.11	0.00	0.00	0.85	0.28	0.21	3.21	4.26
Total PUFA	**0.15**	**0.05**	**0.00**	**0.85**	**0.33**	**0.26**	**3.77**	**4.97**
20:4 (AA)	0.01	0.01	0.04	0.00	0.00	0.00	0.12	0.12
20:5 (EPA)	0.02	0.03	0.13	0.56	0.02	0.02	0.23	0.36
22:6 (DHA)	0.06	0.22	0.34	2.72	0.18	0.18	1.80	3.25
Total HUFA	**0.09**	**0.26**	**0.51**	**3.28**	**0.20**	**0.20**	**2.15**	**3.73**
Total FA	**0.56**	**0.69**	**1.27**	**8.27**	**1.00**	**0.75**	**8.85**	**12.72**
N	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**

The principal component analyses of organisms of both species from the four aquacultures are represented in Figure 3.4.1, and show quite clearly that gilthead seabream organisms reared in aquacultures S1 and S2 (GsS1 and GsS2, respectively) present higher docosadienoic acid when compared to the other seabream individuals (seabream reared in aquacultures M1 and M2, represented as GsM1 and GsM2, respectively). European seabass reared in aquaculture S2 (EsS2) present essentially higher content of DHA compared to their conspecifics (seabass reared in aquacultures M1, M2 and S2, represented as EsM1, EsM2 and EsS1, respectively).

A closer observation of the saturated fatty acid profile (Figure 3.4.2) of the individuals tested revealed that GsS2 individuals stand out from the rest of the groups tested due to their higher palmitic acid content; GsS1 generally presented a higher stearic acid content compared to their conspecifics. Concerning European seabass groups, EsS2 organisms showed higher content in stearic acid than other fish from the same species.

The PCA for unsaturated fatty acid profiles (Figure 3.4.3) revealed that gilthead seabream organisms from the aquacultures in the Sado estuary stand out from their conspecifics reared in the Mondego estuary especially due to their higher docosadienoic acid content. European seabass individuals from aquaculture M2 presented higher DHA content compared to their conspecifics from the other three aquacultures.

PCA for the omega-3 HUFA EPA and DHA (Figure 3.4.4) suggests that, in general, gilthead seabream organisms reared in the Sado estuary stand out from their conspecifics reared in the Mondego estuary due to their higher EPA and DHA content; the similar is noticed for EsS2 organisms, which seem to present higher EPA and DHA content than the other seabass specimens, and for EsS1 organisms, the second European seabass group with higher EPA and DHA content.

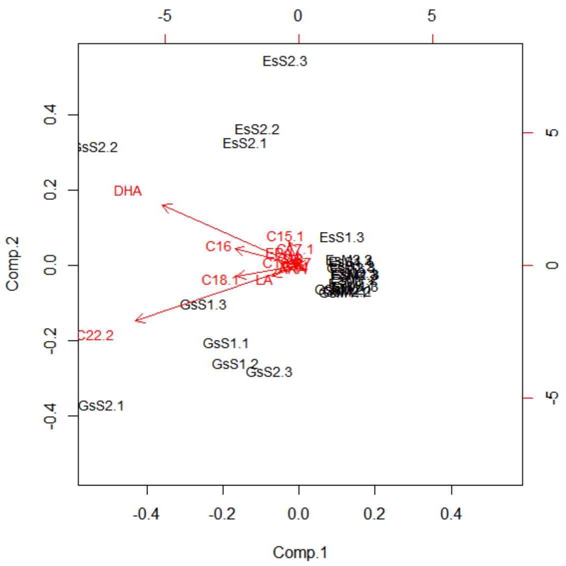


Figure 3.4.1 – Principal component analysis of the total fatty acids content of the organisms analysed. EsM1.1 to EsM1.3, EsM2.1 to EsM2.3, EsS1.1 to EsS1.3 and EsS2.1 to EsS2.3: studied European seabass individuals from aquacultures M1, M2, S1 and S2, respectively. GsM1.1 to GsM1.3, GsM2.1 to GsM2.3, GsS1.1 to GsS1.3 and GsS2.1 to GsS2.3: studied gilthead seabream individuals from aquacultures M1, M2, S1 and S2, respectively.

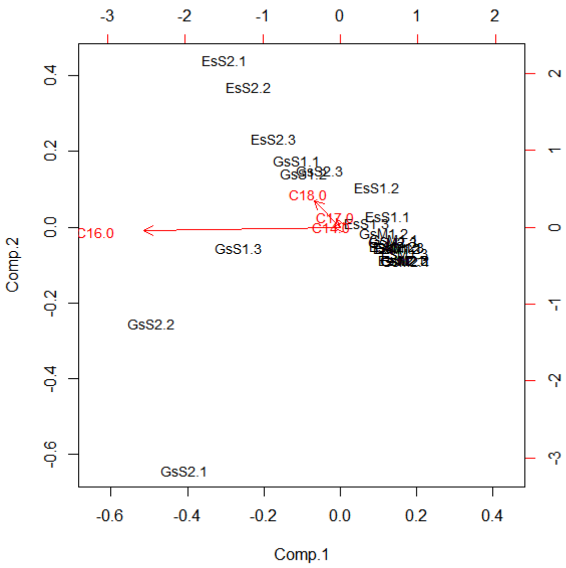


Figure 3.4.2 – Principal component analysis of the saturated fatty acids content of the organisms analysed. EsM1.1 to EsM1.3, EsM2.1 to EsM2.3, EsS1.1 to EsS1.3 and EsS2.1 to EsS2.3: studied European seabass individuals from aquacultures M1, M2, S1 and S2, respectively. GsM1.1 to GsM1.3, GsM2.1 to GsM2.3, GsS1.1 to GsS1.3 and GsS2.1 to GsS2.3: studied gilthead seabream individuals from aquacultures M1, M2, S1 and S2, respectively.

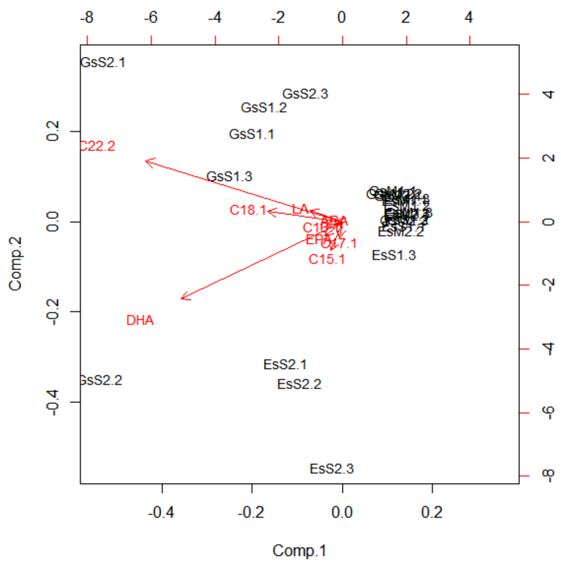


Figure 3.4.3 – Principal component analysis of the unsaturated fatty acids content of the organisms analysed. EsM1.1 to EsM1.3, EsM2.1 to EsM2.3, EsS1.1 to EsS1.3 and EsS2.1 to EsS2.3: studied European seabass individuals from aquacultures M1, M2, S1 and S2, respectively. GsM1.1 to GsM1.3, GsM2.1 to GsM2.3, GsS1.1 to GsS1.3 and GsS2.1 to GsS2.3: studied gilthead seabream individuals from aquacultures M1, M2, S1 and S2, respectively.

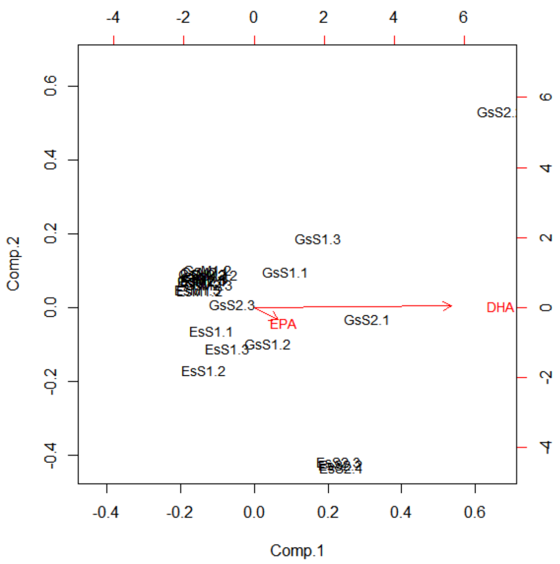
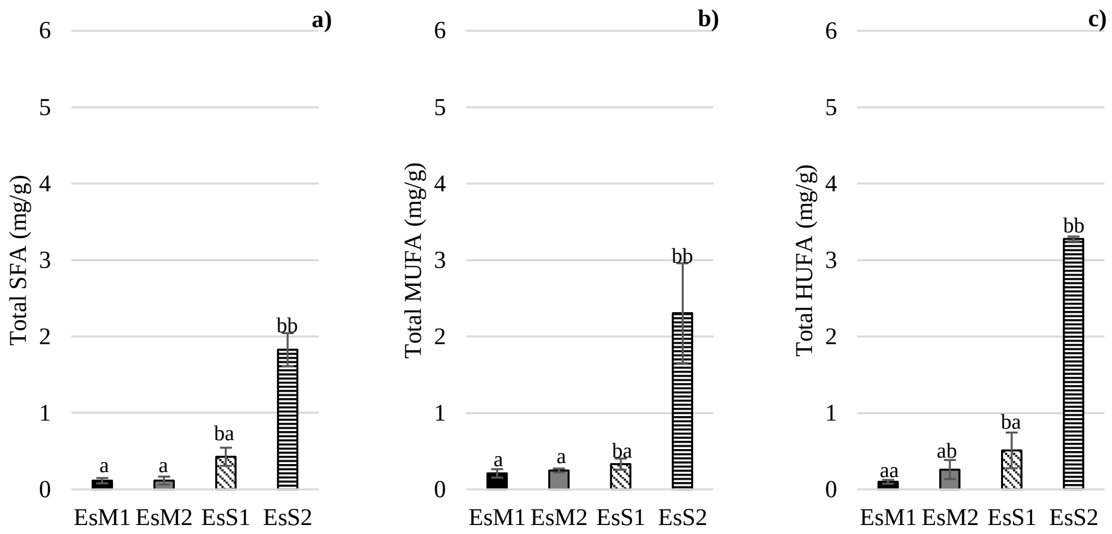


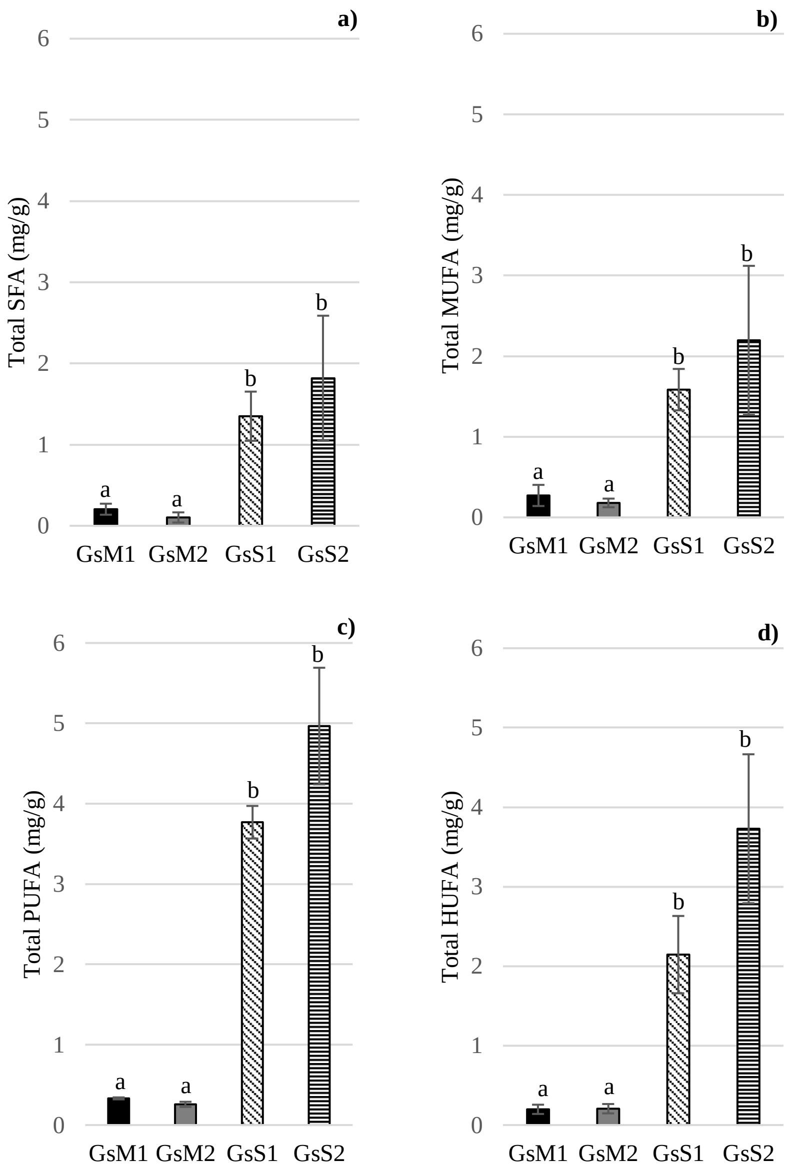
Figure 3.4.4 – Principal component analysis of EPA and DHA content of the organisms analysed. EsM1.1 to EsM1.3, EsM2.1 to EsM2.3, EsS1.1 to EsS1.3 and EsS2.1 to EsS2.3: studied European seabass individuals from aquacultures M1, M2, S1 and S2, respectively. GsM1.1 to GsM1.3, GsM2.1 to GsM2.3, GsS1.1 to GsS1.3 and GsS2.1 to GsS2.3: studied gilthead seabream individuals from aquacultures M1, M2, S1 and S2, respectively.

Analysis of variance testing differences between fatty acid groups in European seabass individuals showed significant differences for total SFA (Figure 3.4.5a), MUFA (Figure 3.4.5b) and HUFA (Figure 3.4.5c) content between estuaries (F=129.80, F=31.94 and F=416.20, respectively, p-value < 0.05 in all cases), with fish from the aquacultures in the Sado estuary showing overall higher contents of the mentioned fatty acid classes when compared to conspecifics reared in the Mondego estuary; organisms cultured in aquaculture S2 presented the highest content of all three fatty acid categories among the European seabass groups. Differences between aquacultures within each estuary were also found for SFA, MUFA and HUFA contents but only between aquacultures S1 and S2 (F= 66.62, F=26.82 and F= 354.10, p-value< 0.05 in all cases). No differences were found between *D. labrax* specimens either between estuaries or aquacultures from the same estuary for total PUFA content (between estuaries F=2.319, aquacultures within the same estuary F=4.039, p-value > 0.05 in both cases).



Figures 3.4.5 a), b) and c) – Total SFA, MUFA and HUFA content, respectively, of European seabass from the four studied aquacultures. Letters above the bars were used to indicate the statistically significant differences among groups; the first letter (or sole letter) refers to the differences (or absence of differences) between estuaries; the second letter refers to the differences found between fish from different aquacultures within the same estuary. Values are expressed in mean ± SD.

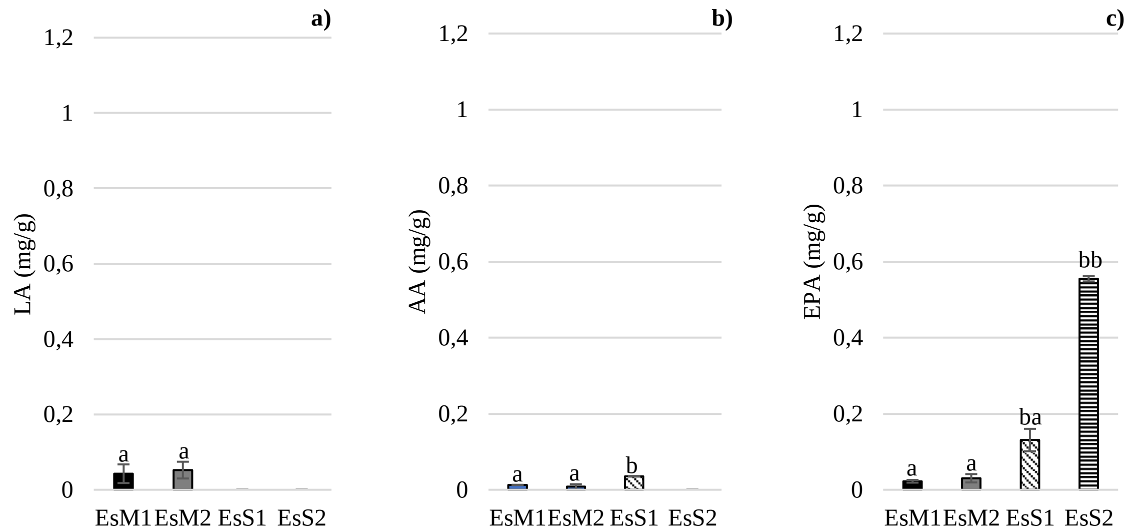
For gilthead seabream, the statistical tests showed significant differences in fish from different estuaries for SFA, MUFA, PUFA and HUFA total content (respectively, F=39.640, F=21.469, F=43.926 and F=7.588, p-values < 0.05 in all cases); the results are represented in Figures 3.4.6a, b, c and d, respectively. In general, organisms from the Sado estuary presented higher content of all four fatty acid categories when compared to the fish reared in the Mondego estuary. However, no differences were found between aquacultures within the same estuary for any of the fatty acid groups in gilthead seabream (F=1.108, F=0.742, F=0.952 and F=0.894 for SFA, MUFA, PUFA and HUFA content, respectively, p-value > 0.05 in all cases). Although fish from aquacultures S1 and S2 presented higher contents of all fatty acid categories tested, the relative abundance of each fatty acid category from the total FA content of fish was similar for all the groups tested.

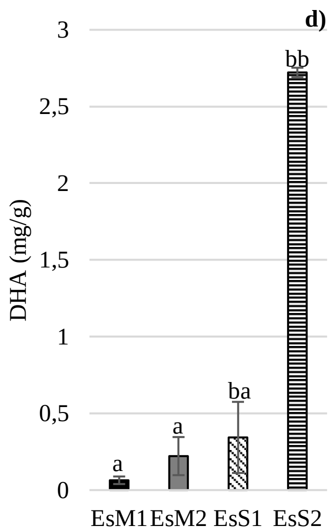


Figures 3.4.6 a), b), c) and d) – Total SFA, MUFA, PUFA and HUFA content, respectively, of gilthead seabream from the four studied aquacultures. Letters above the bars were used to indicate the statistically significant differences among groups; the first letter (or sole letter) refers to the differences (or absence of differences) between estuaries; the second letter refers to the differences found between fish from different aquacultures within the same estuary. Values are expressed in mean ± SD.

Due to their importance for human metabolism and health, the PUFA linoleic acid and the HUFA arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid contents were also submitted to analysis of variance to assess if the set of factors previously mentioned to which the organisms were subjected to during their development had a significant influence on the accumulation of these particular fatty acids in the fish analysed.

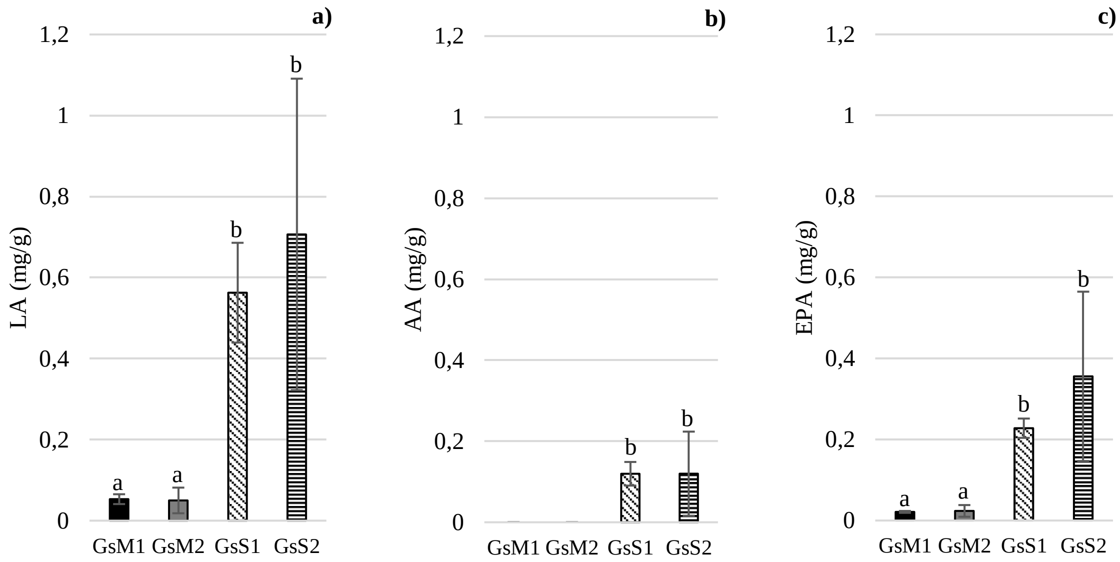
Regarding *D. labrax* specimens, significant differences were found in the accumulation of the four mentioned fatty acids' content between conspecifics from the different estuaries ($F=1147.5$, $F=328.00$, $F=24.320$ and $F=13.74$ for EPA, DHA, LA and AA content, respectively, p-value < 0.05 in all cases). Differences between conspecifics from aquacultures within the same estuary were found for EPA content between EsS1 and EsS2 organisms ($F=514.8$, p-value < 0.05), with fish from aquaculture S2 presenting higher EPA content than EsS1 organisms; for DHA ($F=241.3$, p-value < 0.05) and AA content (F=80.77, p-value < 0.05), differences were found between fish from aquacultures S1 and S2, as only EsS1 individuals presented AA content in their composition. Only the organisms reared in the aquacultures in the Mondego estuary presented LA content, with fish from both aquacultures presenting similar quantities of the referred fatty acid (Figure 3.4.7a), thus explaining the significant differences between estuaries. AA was the HUFA present in lower concentrations in the European seabass organisms studied, and organisms from aquaculture S2 did not contain this fatty acid in their profile (Figure 3.4.7b). Individuals from aquacultures in the Sado estuary presented the higher EPA content when compared to fish reared in the Mondego estuary and fish from aquaculture S2 presented the highest EPA content of all European seabass groups studied (Figure 3.4.7c). DHA was the most abundant FA in all European seabass organisms tested and organisms from the Sado estuary were the ones showing the highest content, with organisms from aquaculture S2 standing out from the other groups (Figure 3.4.7d).

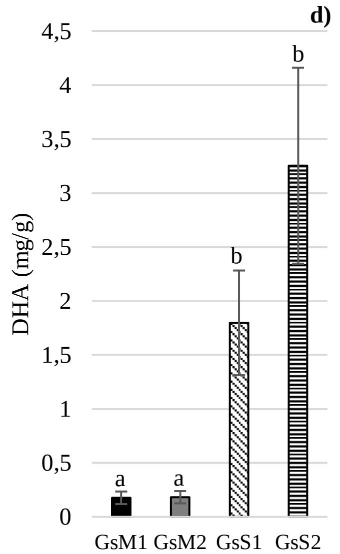




Figures 3.4.7 a), b), c) and d) – Linoleic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content of European seabass from the four aquacultures studied, respectively. Letters above the bars were used to indicate the statistically significant differences among groups; the first letter (or sole letter) refers to the differences (or absence of differences) between estuaries; the second letter refers to the differences found between fish from different aquacultures within the same estuary. Values are expressed in mean ± SD.

The fatty acid content of EPA, DHA, LA and AA determined for *S. aurata* showed significant differences between conspecifics among estuaries (F=19.581, F=9.667, F=24.858 and F=14.8 for EPA, DHA, LA and AA content, respectively, p-value < 0.05 in all cases), but no differences were found for the content of any of the fatty acids in fish from different aquacultures within each estuary (F=1.105, F=0.931, F= 0.378 and F=0.0 for EPA, DHA, LA and AA content, respectively; p-value > 0.05 in all cases). LA content was higher in organisms reared in the Sado estuary when compared to the organisms reared in the aquacultures in the Mondego estuary (Figure 3.4.8a), presenting even higher concentrations than for EPA content (Figure 3.4.8c). Only the organisms reared in the aquacultures from the Sado estuary presented AA (Figure 3.4.8b), and it was the FA that showed the lowest content for these groups of fish. EPA content in gilthead seabream organisms was higher in organisms reared in the Sado estuary, with fish from aquaculture S2 presenting the highest EPA content (Figure 3.4.8c), similar to the observed for European seabass. DHA was the most abundant fatty acid in gilthead seabream organisms, with fish from the Sado estuary showing the highest contents, and the fish reared in aquaculture S2 presenting the highest content of all the groups analysed, although no significant differences were found when compared to organisms reared in aquaculture S1 (Figure 3.4.8d).





Figures 3.4.8 a), b), c) and d) – Linoleic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content of gilthead seabream from the four aquacultures studied, respectively. Letters above the bars were used to indicate the statistically significant differences among groups; the first letter (or sole letter) refers to the differences (or absence of differences) between estuaries; the second letter refers to the differences found between fish from different aquacultures within the same estuary. Values are expressed in mean ± SD.

Free sugar content

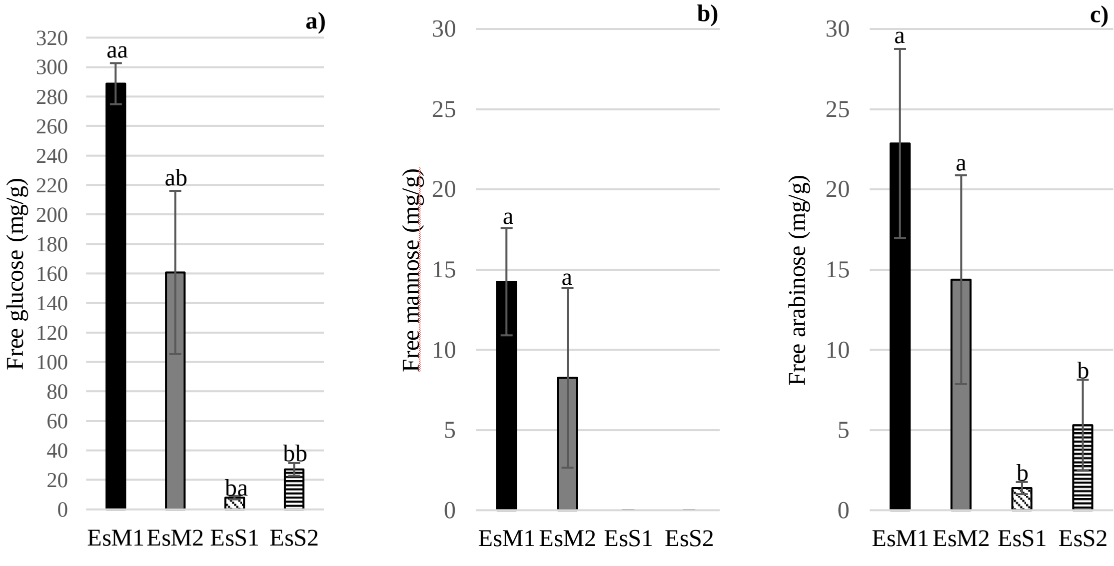
Free sugar quantification results of the fish groups analysed are represented in Table 3.4.4.

Table 3.4.4 – Free sugars arabinose, mannose and glucose content (mg/g of fish muscle tissue) in the fish groups analysed; values are expressed in mean (±SD).

	D. labrax				*S. aurata*			
	M1	**M2**	**S1**	**S2**	**M1**	**M2**	**S1**	**S2**
Arabinose	22.86 (±9.46)	14.37 (±6.51)	1.38 (±0.38)	5.30 (±2.84)	3.73 (±0.89)	4.95 (±3.08)	1.10 (±1.21)	0.78 (±0.37)
Mannose	14.24 (±3.34)	8.26 (±5.61)	0.00	0.00	2.65 (±0.91)	7.07 (±2.11)	0.00	0.00
Glucose	255.26 (±19.70)	179.16 (± 78.27)	7.98 (±1.13)	27.14 (±4.32)	112.04 (±46.20)	147.77 (±13.42)	27.25 (±19.77)	15.73 (±9.65)

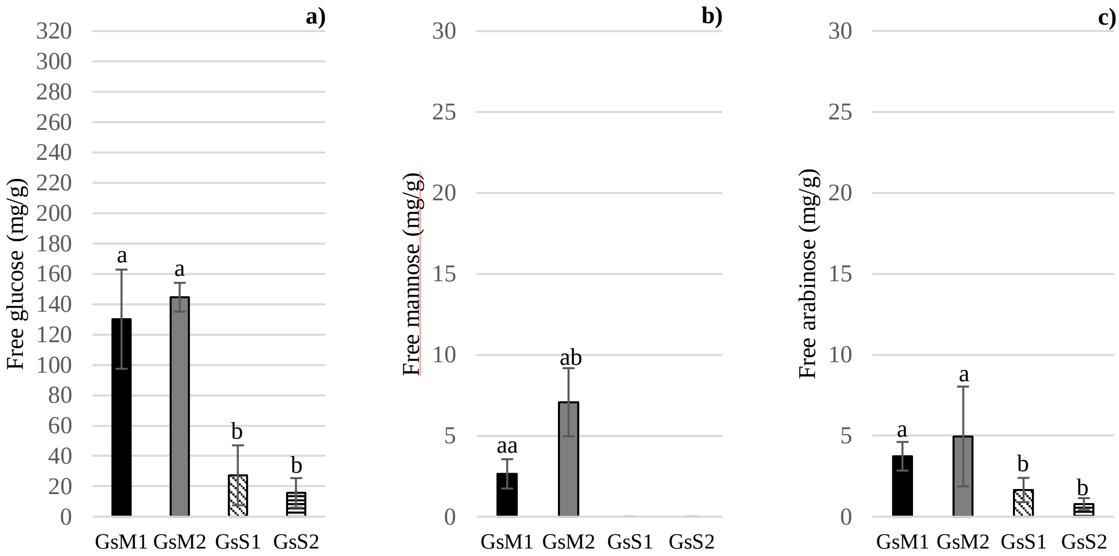
Fish reared in the Mondego estuary presented considerably higher content in all the free sugars identified than the fish from the aquacultures in the Sado estuary, being worth noticing that mannose was only identified in the fish from aquacultures M1 and M2 (Figures 3.4.9 a), b) and c)), and glucose being the most abundant monosaccharide in the samples analysed.

Analysis of variance of free sugar content in European seabass showed significant differences in the content of arabinose, mannose and glucose between estuaries (respectively, F=19.997, F=35.645 and F=157.21, p-value < 0.05 in all cases), and differences between aquacultures within the same estuary were found for glucose content (F=15.35, p-value <0.05) both between aquacultures M1 and M2 and aquacultures S1 and S2 (Figure 3.4.9 a)).



Figures 3.4.9 a), b) and c) – Free glucose, mannose and arabinose total content in mg of free sugar per g of fish muscle tissue in European seabass reared in the four aquacultures. Letters above the bars were used to indicate the statistically significant differences among groups; the first letter (or sole letter) refers to the differences (or absence of differences) between estuaries; the second letter refers to the differences found between fish from different aquacultures within the same estuary. Values are expressed in mean ± SD.

Gilthead seabream reared in the aquacultures in the Mondego estuary presented higher content of all three free sugars when compared to the fish reared in the Sado estuary, with mannose only found in the organisms reared in the Mondego estuary (Figures 3.4.10 a), b) and c)), similar to the observed in European seabass specimens. Gilthead seabream analysis presented significant differences in the three sugars analysed between estuaries (for arabinose content F=10.711, for mannose content F=53.86 and for glucose content F=98.187, p-value < 0.05 in all cases), with differences in organisms from different aquacultures within the same estuary only found for mannose content (F=11.16, p-value<0.05).



Figures 3.4.10 a), b) and c) - Free glucose, mannose and arabinose total content in mg of free sugar per g of fish muscle tissue in gilthead seabream reared in the four aquacultures. Letters above the bars were used to indicate the statistically significant differences among groups; the first letter (or sole letter) refers to the differences (or absence of differences) between estuaries; the second letter refers to the differences found between fish from different aquacultures within the same estuary. Values are expressed in mean ± SD.

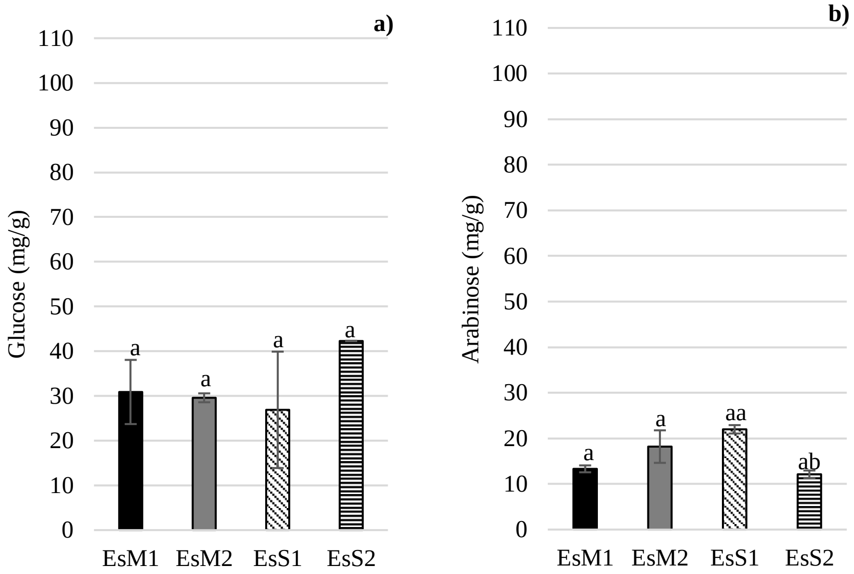
Polysaccharide content

Polysaccharide content was determined based on the monosaccharides present in the samples after hydrolysis. The main residues identified in all samples were mainly glucose. Some samples also showed the presence of arabinose and minor contents of mannose and galactose (Table 3.4.5).

Table 3.4.5 – Content of polysaccharides monomers (mg/g) in the fish groups analysed; values are expressed in mean (±SD).

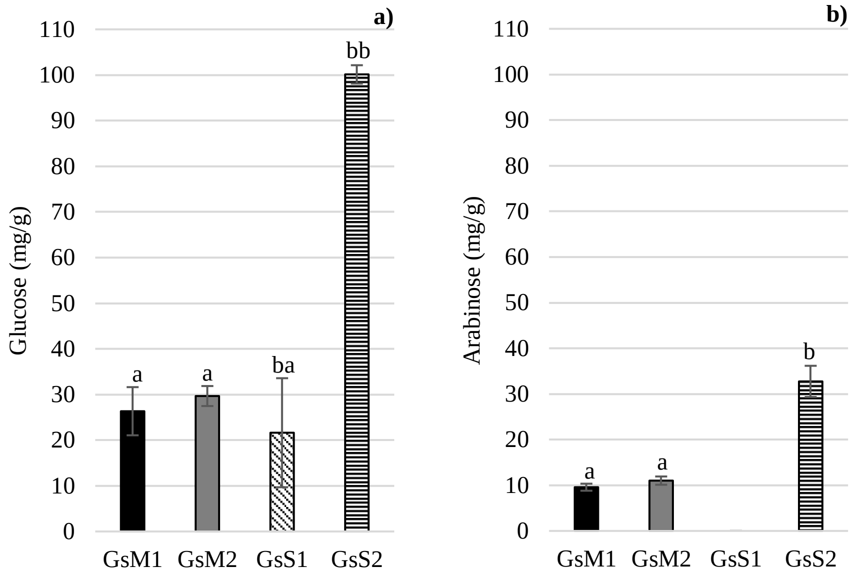
	D. labrax				***S. aurata***			
	M1	**M2**	**S1**	**S2**	**M1**	**M2**	**S1**	**S2**
Arabinose	13.32 (±0.76)	18.20 (±3.57)	21.99 (±0.92)	12.13 (±0.82)	9.58 (±0.77)	11.02 (±0.91)	0.00	32.76 (±3.42)
Mannose	1.31 (±0.52)	1.33 (±0.04)	0.00	0.00	0.82 (±0.18)	1.37 (±0.16)	0.00	0.00
Galactose	0.00	0.67 (±0.14)	0.00	0.00	0.28 (±0.07)	0.33 (±0.08)	0.00	0.00
Glucose	30.87 (±7.17)	29.59 (±1.00)	26.90 (±13.01)	42.29 (±0.01)	26.34 (±5.28)	29.67 (±2.19)	21.63 (±11.94)	100.14 (±2.00)

Glucose was the most abundant monosaccharide observed in polysaccharide analysis (Figure 3.4.11 a)), followed by arabinose (Figure 3.4.11 b)). Mannose and galactose were present in much lower concentrations (below 2 mg/g of fish muscle tissue of both free sugars) when compared to the other sugars in the European seabass specimens analysed (with arabinose concentrations ranging from 13.32 to 21.99 mg/g and glucose concentration from 26.90 to 42.29 mg/g of muscle tissue) as shown in Table 3.4.5; contrary to the observed for arabinose and glucose contents, mannose was only found in *D. labrax* specimens reared in aquacultures M1 and M2, whereas galactose was only present in the tissue of European seabass reared in aquaculture M2. Significant differences amongst European seabass between the two estuaries were found for mannose (F= 78.099, p-value< 0.05) and galactose (F= 70.33, p-value< 0.05) content, as the two monomers were only found in fish reared in the aquacultures on the Mondego estuary, with galactose only present in EsM2 organisms. Differences between conspecifics from aquacultures within the same estuary were also found for arabinose content (F=24.493, p-value < 0.05), and for galactose (F=70.33, p-value < 0.05), given that the monomer was only present in EsM2 organisms.



Figures 3.4.11 a) and b) – Polysaccharide monomers glucose and arabinose total content in mg of sugar per g of fish muscle tissue in European seabass reared in the four aquacultures. Letters above the bars were used to indicate the statistically significant differences among groups; the first letter (or sole letter) refers to the differences (or absence of differences) between estuaries; the second letter refers to the differences found between fish from different aquacultures within the same estuary. Values are expressed in mean ± SD.

Concerning gilthead seabream organisms, glucose was also the most abundant monomer, with GsS2 organisms presenting a considerably higher glucose content than the remaining groups (Figure 3.4.12va)); arabinose was the second most abundant monomer (Figure 3.4.12 b)), although it was absent in GsS1 fish. Mannose and galactose were, similarly to the observed in seabass organisms, only present in fish reared in aquacultures M1 and M2 and in very low concentrations. Significant differences were found between estuaries for all four polysaccharide components tested (for arabinose content F= 33.84, for mannose content F= 244.68, for galactose F= 107.705 and for glucose content F= 72.35, p-value< 0.05 in all cases). Differences between aquacultures within estuaries were found for i) arabinose (F= 246.35, p-value< 0.05), especially due to GsS2 organisms, that present the highest arabinose content compared to the absence of this sugar in fish from aquaculture S1; ii) mannose (F=15.03, p-values< 0.05), with organisms from GsM2 presenting higher content than their conspecifics from aquaculture M1; iii) glucose content (F=103.30, p-value< 0.05), with GsS2 organisms presenting much higher glucose content than GsS1, as mentioned above.



Figures 3.4.12 a) and b) - Polysaccharide monomers glucose and arabinose total content in mg of sugar per g of fish muscle tissue in gilthead seabream reared in the four aquacultures. Letters above the bars were used to indicate the statistically significant differences among groups; the first letter (or sole letter) refers to the differences (or absence of differences) between estuaries; the second letter refers to the differences found between fish from different aquacultures within the same estuary. Values are expressed in mean ± SD.

3.5. Discussion

Fish are known to be good sources of animal protein, being estimated that wild seabass and seabream contain around 20g of protein per 100g of muscle, as reported by a wide number of scientific panels of organizations worldwide (e.g. ESFA, 2012; USDA, 2016) and confirmed by various studies assessing protein content of wild seabass (e.g. Alasalvar et al., 2002; Fuentes et al., 2010) and seabream (Grigorakis, et al., 2002; Senso et al.,2007). As carnivorous fish, seabass and seabream require high amounts of protein in their diet, and thus aquaculture commercial feeds often contain more than 40% of crude protein to match such needs (Huss, 1988; Santinha et al., 1996; Peres & Oliva-Teles, 1999; Cahu et al., 2004). As the organoleptic composition of cultured fish is controlled by the composition of the provided feed, the much higher protein content of fish from both species analysed in the present work, ranging from nearly 41 to 53g of protein per 100g of fish muscle tissue in *D. labrax* and from 41 to 63g per 100g in *S. aurata*, compared to estimations for wild organisms, was thought to be due to the high protein content of the feed provided (from 40 to 44% of the total feed composition). However, similar studies assessing the macronutrient composition of cultured fish using feed with equal or higher protein composition (e.g. Orban et al., 1996; Alasalvar et al., 2001; Alasalvar et al., 2002; Grigorakis et al., 2002; Fuentes et al., 2010), found the protein content of farmed and wild fish to be quite similar, and around 20g per 100g of fish tissue muscle. Possible explanations for the disparity of the results obtained in the present study to the obtained in similar studies may be on the rearing methods applied for production, as *D. labrax* and *S. aurata* were reared in net cages offshore instead of estuarine transitional waters as referred by Alasalvar et al. (2002) and Fuentes et al. (2010), and Alasalvar et al. (2001), respectively. In fact, the study conducted by Orban et al. (2000) showed that different rearing systems have a significant influence on the organoleptic composition of cultured fish, which could have also been the case here. Although there is no certainty about what may have caused such differences, the results obtained in the present study provide some evidence of the influence that a set of extrinsic factors, such as environmental conditions, stress in the tanks, quantity and composition of food provided, among others, that fish are subjected to during their development, have in the organoleptic composition of fish from the same species when reared in different conditions.

The fatty acid profile of cultured fish reflects the composition in fatty acids of the feed supplied, as shown by many studies of different fish species, such as Atlantic salmon (e.g. Hardy et al., 1987), brook charr (e.g. Guillou et al., 1995), freshwater fish (Henderson and Tocher, 1987), gilthead seabream (Grigorakis et al., 2002; Izquierdo et al., 2003, 2005), European seabass (Alasalvar et al., 2002; Izquierdo et al., 2003; Fuentes et al., 2010). Thus, fatty acid profiles between cultured fish may vary according to differences in composition of the feed provided and other sources organisms might feed on.

Palmitic acid is commonly the most abundant saturated fatty acid observed in various marine fish species, such as turbot (Sérot et al., 1998), gilthead seabream (Grigorakis et al., 2002; Mnari et al., 2007; Sağglık et al, 2003) and European seabass (Sağglık et al., 2003; Orban et al., 2002). The percentage of SFA of the total fatty acid content in European seabass (close to 20%) in organisms from aquacultures M1, M2 and S2, was lower than the value found for cultured and wild conspecifics in similar studies and only the fish reared in aquaculture S1 presented a SFA percentage (around 33%) consistent with values found for farmed seabass in similar studies (Delgado et al., 1994; Alasalvar et al., 2002; Orban et al., 2002; Fuentes et al., 2010). A similar pattern occurred with gilthead seabream organisms studied, which presented SFA percentages generally lower (around 15 to 21%) than the observed in similar studies (Grigorakis et al., 2002; Mnari et al., 2007). Concerning the actual content of SFA of the organisms studied, European seabass organisms presented much lower content of this fatty acid class, ranging between 0.11 and 1.83mg/g of fish muscle tissue, when compared to estimations for wild conspecifics, which are around 5,11mg/g of fish muscle tissue (USDA, 2016). The similar was observed for the gilthead seabream groups, which present total SFA contents ranging between 0.11 and 1.82 mg/g, whereas the estimated for wild organisms is about 6.40mg/g of fish muscle tissue (USDA, 2016).

Regarding MUFAs content, European seabass reared in aquacultures M1 and M2 showed similar results to the observed by other authors (Lanari et al., 1999; Alasalvar et al., 2002; Orban et al., 2002; Fuentes et al., 2010), with MUFAs representing over 35% of the total fatty acid content in farmed fish. The results obtained for the same species reared in the Sado estuary were closer to the estimated for wild conspecifics, with MUFAs percentage accounting for about 20% of total fatty acid content (Alasalvar et al., 2002; Fuentes et al., 2010). MUFAs percentage was also lower than the values found in similar studies for farmed gilthead seabream, being around 18% for organisms reared in aquacultures S1 and S2 and between 24 and 26% in organisms from aquacultures M1 and M2, compared to about 30% in the same species assessed in similar studies (Grigorakis et al., 2002; Mnari et al., 2007). Oleic acid is usually the most abundant monoenoic fatty acid in cultured fish, a pattern that has also been identified in other studies assessing farmed fish such as turbot (Sérot et al., 1998), European seabass (Alasalvar et al., 2002; Fuentes et al., 2010; Sağglık et al., 2003) and gilthead seabream (Mnari et al., 2007), and confirmed by the results obtained for *D. labrax* from aquacultures M1, M2 and S1 and *S. aurata* from all aquacultures. The high content of oleic acid found especially in cultured fish species has been reported by some authors to be due to the dominance of the mentioned fatty acid in feed for aquaculture production (Pagliarani et al. 1986; Grigorakis et al., 2002), as it occurs in many animal and vegetable oils, used in feed production.

PUFA content of the farmed fish studied was rather distinct between aquacultures. Comparing to similar studies assessing the fatty acid profile of farmed seabass (e.g. Alasalvar et al., 2002; Fuentes et al., 2010), PUFA percentages in EsM2 and EsS2 organisms (around 7 and 10%) were lower than the values found in those studies, which registered PUFA percentages between 13 and 17% of the total fatty acid content; however, similar results to the ones obtained in the present work were found by Orban et al. (2002). On the other hand, EsM1 organisms presented a considerably higher percentage (around 27%) of PUFA compared to EsM2, EsS1and EsS2 organisms and to the fish assessed in the mentioned studies. The PUFA percentage of total fatty acid content in gilthead seabream, accounting for about 33 to 40% of the total fatty acid composition, was also much higher than the values found in other studies, such as in Grigorakis et al (2002) (around 13%) and in Mnari et al. (2002) (around 7%). PUFA content of the studied fish comprehended only two fatty acids – linoleic acid and docosadienoic acid – differing considerably from the similar studies mentioned, where, in general, fish species presented other polyenoic fatty acids besides LA and did not have docosadienoic acid in their composition. Although LA is usually the most abundant polyunsaturated fatty acid found in farmed seabass (Alasalvar et al., 2002; Orban et al., 2002; Fuentes et al., 2010) and farmed seabream (Grigorakis et al., 2002; Sağglık et al., 2003; Mnari et al., 2007), it was the second most abundant in the fish species here studied, following docosadienoic acid. High amounts of LA are not expected to be found in wild fish, given that it is mainly present in plant oils, which is the reason the fatty acid appears in higher quantities in farmed fish, due to the fact that commercial feeds usually contain such sources of linoleic acid (Owen et al., 1975; Sérot et al., 1998; Grigorakis 2007).

Only EsM1 organisms presented a total HUFA percentage (around 16%) similar to the reported for farmed organisms in other studies, such as the ones conducted by Fuentes et al. (2010), with fish presenting about 15% of total FA; the other studied European seabass groups presented higher contents (from 38 to 40% of total FA) than the registered for farmed conspecifics in similar studies (Orban et al., 2002; Fuentes et al., 2010), and closer to the values found in wild organisms. Gilthead seabream, on the other hand, presented HUFA percentages (from 20 to 29% of total FA) similar to the obtained by other authors for farmed seabream (Grigorakis et al., 2002), but lower than the values found in other studies (Mnari et al., 2007). DHA and EPA were the HUFA presenting the highest contents in all organisms, results that are consistent with previous studies stating that these are the main HUFA found in fish (Alasalvar et al., 2001; Alasalvar et al, 2002; Grigorakis et al, 2002; Grigorakis, 2007; Mnari et al., 2007).

Regarding the actual content on total unsaturated fatty acids, comprehending MUFA, PUFA and HUFA contents, of the fish species studied, the values obtained for European seabass specimens were much lower than the estimated for wild consecifics; EsM1, EsM2 and EsS1 presented a total unsaturated FA content lower than 1mg/g of fish tissue and EsS2 organisms, although presenting 6,44mg/g, remained far from the estimated for the species – about 12mg/g of fish fillet (USDA, 2016). GsM1 and GsM2 also contained a total of unsaturated fatty acids below 1mg/g of fish muscle tissue and GsS1 organisms presented around 8mg/g of total unsaturated fatty acid in muscle tissue samples; only GsS2 specimens presented a value that surpassed the estimated value for wild conspecifics (around 10mg/g, according to the USDA (2016)), accounting for about 11mg/g of fish fillet.

The differences between the results obtained in the present study and the obtained in the mentioned studies regarding the fatty acid profiles of fish of the same species could be mainly due to differences in the fatty acid profile of the feeds provided and influence of the different rearing sites and production methods applied, as the fish studied by the mentioned authors were reared in offshore facilities, instead of transitional waters, and thus subjected to a whole different set of extrinsic parameters influencing their development.

Regarding the differences found in the content of the different fatty acid classes and specifically in EPA, DHA, AA and LA contents between the seabass and seabream conspecifics studied could not have been due to feed composition, as the same feed was provided in three of the four aquacultures and no differences were found in the content of the named fatty acids between fish from aquacultures M1 and M2. Different water qualities, biological communities and organic matter entering the rearing ponds or the different feeding methods may have influenced differently the fatty acid accumulation in fish; for instance, the set of extrinsic factors on the Sado estuary seem to have been, in general, more suited for the accumulation of every fatty acid group (apart from EsM1 organisms) in both species, in EPA and DHA accumulation in European seabass and gilthead seabream and for the accumulation of AA and LA in gilthead seabream; for European seabass, on the other hand, accumulation of LA was higher in organisms reared in aquacultures M1 and M2. Differences found between fish among aquacultures within the same estuary were only found for European seabass reared in aquacultures S1 and S2 for EPA, DHA and AA content; although the same feed was provided and the estuary was the same, aquacultures were placed in areas subjected to different human pressures, which may have caused local differences in water quality. Aquaculture S1 was placed in an area closer to an industrial park, with high pollutant input to the water; aquaculture S2 was placed in an area with more agricultural fields, and thus the nutrient input from water runoff could have been higher in the water used for aquaculture S2, which could explain partly the differences found; moreover, stress in tanks and the feeding methods, mechanical in aquaculture S1 and manual in S2 may have also contributed for such differences between conspecifics. However, such different pressures did not have a significant impact on the accumulation of the four fatty acids in gilthead seabream.

Glycogen is the only polysaccharide of animal origin, stored in muscle and liver of animals; other polysaccharides appearing in fish are of dietary origin, usually from plants (FAO, 2016). Glycogen is composed by glucose monomers, explaining the higher levels of glucose compared to other monosaccharides content in the present polysaccharide analysis. Arabinose is present in polysaccharides from vegetable origin, as wheat and carob germ. Mannose is an important component of plant and bacterial polysaccharides, and human glycoproteins and glycolipids (Herman, 1971; Etchison & Freeze, 1997); amongst main sources of mannose are, for example, carob gum (Herman, 1971; Van Immerseel et al., 2002; Hu et al., 2016), which are present in the commercial feeds provided to the fish studied, explaining the appearance of the referred monosaccharide in the polysaccharide analysis. Feeds used in the aquaculures contained the mentioned possible sources of arabinose and mannose and thus its presence in fish muscle composition is most probably due to feed digestion. The monomers referred may also be present as free monosaccharides, collectively mentioned as free sugars, which may also come from feeding and degradation of more complex molecules, as glycoproteins.

Differences in free glucose content between European seabass from aquacultures M1 and M2 could have been due to the fact that the cultures are placed in areas that can be subjected to different pressures which, although not thought to be as marked as in the aquacultures in the Sado estuary, still supports the assumption that different rearing sites affect differently the composition of products. Differences in glucose content between aquacultures and S1 and S2 could have been due to its location in an area more subjected to water runoff from agricultural fields, which could potentially bring organic matter of vegetable origin, in which fish may feed on, thus resulting in the observed contents. The differences found in mannose content in gilthead seabream between aquacultures M1 and M2 could have been due to the influence of fish feeding in organic matter entering the rearing ponds, as mannose in fish is of dietary origin; other explanation could have been the different feeding methods applied, as fish from aquaculture M1 are fed manually and fish from aquaculture M2 are fed through a mechanical system.

The differences in polysaccharide composition of fish between the two estuaries may also be potentiated by the different sets of extrinsic factors influencing the rearing sites, for example in terms of organic matter entering the rearing ponds in which fish may have fed on or stress in the tanks, influencing the fish feed intake and digestion, which may have resulted on the differences found.

Galactose is mainly present in mammalian milk, and thus it was not expected to be found in considerable amounts in the analysed fish; however, it is also present in some vegetable sources as soybean, which, although not present in the feeds provided, may have entered the rearing tanks with water renewals to be ingested by fish, thus resulting on the galactose content found, even if in small concentrations.

As commonly referred, we truly are what we eat, which has led to decades of studies regarding human nutrition and understanding the role of dietary macromolecules in our biological processes, on how they influence our development and health.

Dietary carbohydrate, lipid and protein intake are human's main sources of energy and provide essential elements to the functioning and development of the organism (Webster-Gandy, Madden & Holdsworth, 2006). Recommended intake dosages of these macronutrients are calculated to match the nutritional and metabolic needs of human beings, according to age, gender, level of activity, or adapted to one's specific case. The quality of food has therefore been given great importance to, and the demand for high quality products has increased over the years. Fish quality is dependent essentially on its freshness, based on the overall sensory properties of the fish (Martin, 1988) and nutritional value (Grigorakis, 2007).

Recommended daily intake dosages of protein for the average adult have not yet been established for the European population, due to a lack of data that could directly relate protein consumption to health outcomes (ESFA, 2015). Nonetheless, Average Requirement (AR) values for protein intake have been established for European populations. The daily AR of protein has been set in 0,66g/kg of body weight for an adult (Rand, Pellet & Young, 2003; WHO/FAO/UNU, 2007), meaning that an adult weighting around 70kg should consume about 45g of protein daily. It is estimated that in many European countries, the daily protein intake ranges between 59 to 114g/day for adults (EFSA, 2015).

The fish studied present close to 50 g of protein per 100g of muscle tissue, the double or more than the average expected from wild counterparts. Once there are no health outcomes known for protein intake dosages higher than the values advised, this should not be a concern in terms of public health, although attention should continue to be paid.

The obtained results also show that, in a human nutrition view, fish from both species studied supply considerably lower saturated fatty acid content when compared to the total unsaturated fatty acid content, thus supporting the general assumption that fish are good sources of unsaturated fatty acids, especially of the omega-3 series, and contain lower saturated fatty acids than fish from the same species from other origins. According to the USDA (2016), total saturated fatty acid content for wild seabass and seabream species should be around 0.50 and 0.65 grams per 100 grams of total weight, respectively; European seabass and gilthead seabream organisms from all four aquacultures present much lower saturated fatty acids concentrations than the estimated for wild fish. Apart from some species (Ackman, 1989; Nettleton & Exler, 1992), fish present relatively low SFA content, especially lean fish, which is confirmed by the results obtained, and it may then be suggested that the organisms studied do not pose potential health threats caused by high consumption of saturated fatty acids.

Dietary unsaturated fatty acids bring various health benefits, as already mentioned, and so it is proposed that, although no DRVs have been set for European populations, most fat intake should come from sources rich in monounsaturated, polyunsaturated and highly-unsaturated fatty acids, of which fish are a good example (Russo, 2009; EFSA, 2010). Reports of the USDA (2016) refer that wild European seabass specimens contain about 0.42g/100g of total MUFAs and seabream specimens around 0.56g/100g; in terms of combined PUFAs and HUFAs content, wild seabass is expected to contain about

0.74g/100g and gilthead seabream 1.03g/100g in muscle tissue. Although fish from the Mondego estuary present unsaturated fat contents considerably lower than the estimated for wild fish, fish from the Sado estuary in general present values very close to the estimated contents of wild fish.

Although both EPA and DHA can be synthetized from the essential fatty acid α-linolenic acid, the rate at which the conversion takes place and the amounts produced are not sufficient to match human needs, and should thus be obtained from food sources (Tocher & Dick, 2001; Simopoulos, 2009; Nichols et al., 2014). The recommended daily dosage of EPA and DHA combined intake for the European population ranges between 200 to 500 mg (Lee, 2009; EFSA, 2010) and for people with coronary heart disease or other conditions that could benefit from EPA and DHA intake and pregnant or lactating women, the recommended dosage may vary according to authors but it is estimated to be around 1 g per day (Kris-Etherton, Harris & Appel, 2002; Lee, 2009; ESFA, 2010).

Fish are the main source of dietary EPA and DHA intake for humans, however, the progressive replacement of fishmeal in aquaculture feeds with vegetable meals has been proven to lower the fatty acid content of fish (Kissil et al., 2000; De Francesco et al., 2007), essentially in the EPA and DHA content of farmed fish, as vegetable sources are not rich in such fatty acids, usually resulting in their lower concentrations when compared the expected from wild organisms (Olsson et al., 2003; Regost et al., 2003; Izquierdo et al., 2003, 2005; Montero et al., 2005; Mourente et al., 2005).

Although fish of both species from the aquacultures in the Mondego estuary and European seabass from aquaculture S1 presented quite low contents of EPA and DHA, the remaining organisms reared in the Sado estuary presented total HUFAs values within the daily recommended dosage intake for healthy adults.

Carbohydrates are one of humans' main dietary energy sources, being recommended that carbohydrate intake represents 40% of the total daily energy intake in adults (HCN, 2001), estimated to be about 130g of daily intake (Trumbo et al., 2001). Polysaccharides and free sugars are a minor constituent of fish, as already mentioned, and thus fish are not a good source of such nutrients for humans. Nonetheless, fish from aquacultures M1 and M2 of both species seem to provide higher dietary carbohydrate content, especially in terms of free glucose and glycogen, than the fish from aquacultures S1 and S2; mannose content of fish from aquacultures M1 and M2 can also be taken into consideration, as the referred monomer plays an important role in human metabolism for the glycosylation of proteins, although not being an essential nutrient, as it can be produced from glucose (Ichikawa et al., 2014).

In a broad view, the rearing zone had some influence on the general nutritional value of the fish produced, for both species, especially in terms of fatty acid and carbohydrate content. From the consumer point of view, this may come as a disadvantage, as, when looking for a specific product, the consumer trusts that the quality and composition of the product does not depend on its origin and should be close to the assumed quality of wild counterparts.

It would be interesting to pursue the present study in order to comprehensively assess which factors were indeed on the origin of the variability in nutritional content of the fish analysed, which could further develop the knowledge about semi-intensive rearing systems limitations' and search for ways to diminish their impact. The study of the nutritional composition of wild *D. labrax* and *S. aurata* caught along the coast of Portugal could also be interesting to determine if the differences found between the studied fish and estimations for wild conspecifics verify among the Portuguese wild and cultured fish.

Although fish consumption has been widely recommended to maximize the health benefits already stated, recent studies have raised some concerns about the balance between the benefits from fish consumption and the risks associated with their high pollutant contents (Kromhout et al. 2016, Mozaffarian et al., 2010; Barbarossa et al., 2016). Thus, another interesting path to pursue in the future would be to assess the metals and persistent pollutants content that are absorbed by the tissues of the study groups, in order to determine if frequent and long-term consumption of such organisms could potentially cause toxicity issues and health risks to consumers.

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Chapter 4

General Conclusions

Fish aquaculture production is assuming greater importance everyday worldwide, as a means of obtaining healthy animal protein, encouraged by the special interest of the general public in the known health benefits promoted by fish consumption.

The present study highlighted that aquaculture production in Portugal has been following a rising trend in terms of production volume and that most marine fish species with commercial interest reared in the country are produced in semi-intensive rearing systems, where poor control over the characteristics of the surrounding environment is achieved. The study conducted to assess if the nutritional composition of seabass and seabream was dependent on the rearing site, with potentially distinct water quality, environmental parameters, dimensions and stress within the rearing tanks, among others factors, found significant differences in the macronutrient composition between conspecifics reared in the two distinct estuaries and among aquacultures within the same estuary. This may not only pose a disadvantage in a marketing perspective of products, but also in terms of the risks that production farms may be subjected to if the environmental conditions become improper for fish production.

One of the main concerns and constraints raised by consumers about choosing cultured fish over their wild counterparts for consumption is related to the nutritional composition of farmed fish, usually thought to provide fewer nutritional and health benefits, compared to the benefits obtained from wild fish consumption. The analyses conducted in the present study allowed to observe that, in terms of protein supply, the cultured fish studied presented more than twice the content of protein per unit of fish muscle tissue estimated for the species (based on wild organisms' composition); however, whether the supply of this macronutrient is adequate for the nutritional intake of human beings depends on one's specific case. Regarding fatty acid supply, differences were also found between cultured fish and the estimated for wild conspecifics, with the fish studied presenting general lower fatty acid content compared to their wild counterparts. The saturated fatty acid content of the studied fish per unit of muscle tissue was much lower than the estimated for wild specimens, which may be regarded as an advantage in terms of human health; however, the unsaturated fatty acid supply, apart from gilthead seabream reared in aquaculture S2, was also lower than the estimated for wild individuals, especially in fish reared in the aquacultures in the Mondego estuary, which may not be as appealing from a consumer's perspective. However, it is worth noticing that, regarding the supply of EPA and DHA, the results obtained were more promising in terms of the benefits from cultured fish consumption, as the combined content of the two omega-3 fatty acids in European seabass reared in aquaculture S2 and gilthead seabream reared in aquacultures S1 and S2 was within the recommended daily dosages of intake of these fatty acids. Thus, these organisms can be considered good sources of omega-3 fatty acids, similarly to their wild counterparts.

In a world where human population ever-growing demand for fish is leading to the overexploitation and depletion of wild stocks, aquaculture comes as an important means of releasing the current fishing pressure on wild fish populations. However, various significant differences that cultured fish still present compared to their wild counterparts, especially in terms of nutritional composition, as mentioned above, may constitute a constraint against the preference of farmed fish over wild products by consumers. Moreover, the environmental impacts caused by aquaculture production demand legislation and thorough planning of its development, in search for a balance that minimizes the potential negative impacts of the activity, while maximizing the production. At the same time, there is a need for better practises in aquaculture production and the adaptation of rearing techniques to produce fish in both quantity and quality close to the achieved with wild conspecifics.

It would be interesting to further develop the present study, namely through the assessment of the extrinsic factors that influence the organoleptic composition of both species from different sites, which would promote a wider understanding of the limitations of semi-intensive rearing systems. It could also potentiate the search for solutions that would minimize the influence of extrinsic characteristics on the development of fish and enhance the production in semi-intensive systems, while maintaining this production method, that sustains a significant traditional value and some natural influence in an artificial means of food production. Another interesting path to pursue in the future would be to determine the metal and persistent pollutant accumulation on the tissues of the studied species, in order to evaluate if the long-term consumption of the referred fish, in the search for the health benefits they are known to bring, could in fact pose toxicity issues and consequent health risks to consumers.