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Marine hydrolysates as functional nutritional supplements to promote European seabass (*Dicentrarchus labrax*) robustness addressing the European zero-waste strategy

UNIVERSIDADE DO ALGARVE
Faculdade de Ciências e Tecnologia

2023

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Dissertation for the obtention of the master's degree in
Aquaculture and Fisheries – Specialization in Aquaculture

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Declaração de autoria

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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(João Rodrigues)

Acknowledgments

This work was supported by Project "FISHCOLBOOSTER - Desenvolvimento de péptidos colagénicos de peixe em sistema integrado com obtenção de frações de elevado valor para alimentação humana, aquacultura e cosmética" with reference POCI-01-0247-FEDER-049636, and supported by Operational Program for Competitiveness and Internationalization (COMPETE2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

Additionally, João Rodrigues was financed by Fundação Amadeu Dias within the scope of CIIMAR's BYTPlus program.

I would like to thank Prof. Luísa Valente for making the lab and bioterium room available and supplying the necessary materials to make this work possible.

A special thanks to Dr. Cristina Velasco and Dr. Cláudia Aragão for accepting the challenge of being my supervisors. Both were essential by giving scientific support, feedback, and helping whenever needed. Again, thank you for everything!

To all the members of LANUCE who accompanied me through the past months, a sincere thank you! A special appreciation to Tiago Sá and Luciano Santos for their continued help, encouragement, and companionship.

To my friends, a sincere thank you for accompanying me through the years having to handle me at my best and especially at my worst. All of you made me a better and happier person, and I'm so grateful for that.

To my parents and closer family, thank you for always encouraging me to keep moving forward and never give up. You made it possible for me to keep studying so I could achieve higher avenues in life and for that and a lot more… I will be forever indebted to you.

Abstract

Traditionally, fishmeal is the standard dietary protein for aquaculture-produced species, but environmental and economic concerns drive the shift to sustainable-oriented practices. Plant-based proteins are a more sustainable alternative to fishmeal. However, they have drawbacks such as antinutritional factors that affect fish growth, intestinal health, and immunity. Recent research indicates that low dietary inclusion rates of marine protein hydrolysates enhance the nutritional quality of high-vegetable protein diets, positively impacting fish growth and robustness. Thus, in this study, the effects of supplementing a highly vegetable-based commercial diet with 3% blue shark skin hydrolysate on the growth, stress response, and disease resistance of juvenile European seabass were assessed.

The fish growth parameters were mostly unaffected when comparing the experimental (SHARK) to the commercial diet (CTRL), except for a higher protein efficiency ratio (PER) in the CTRL treatment. However, fish showed good acceptance, metabolization, and utilization of the SHARK diet. The whole-body composition showed a higher ash content in the SHARK treatment, likely due to the hydrolysate mineral profile. In response to a stress challenge, fish fed both diets presented a typical increase in glucose, lactate, and cortisol levels. Interestingly, fish fed the SHARK diet showed significantly lower cortisol levels, suggesting better energy utilization. No negative significant impact on animal metabolism was observed. Regarding the immune markers, peroxidase levels were higher in the CTRL treatment after the stress challenge, but other immune indicators remained unaffected by the diet. During the infectious challenge with *Photobacterium damselae* subsp. *piscicida*, both treatments showed similar low cumulative mortality rates.

This study demonstrates that incorporating low levels of blue shark skin hydrolysate into low fishmeal diets does not impact the growth and robustness of juvenile European seabass. This suggests that the hydrolysate is a safe and beneficial ingredient for aquafeeds targeted at this fish species.

Keywords: fish by-products; hydrolysates; bioactive peptides; functional diet; sustainable feed; circular economy

Resumo

É esperado que a população mundial atinja 9,7 mil milhões até 2050, resultando numa procura crescente por alimentos nutritivos, saudáveis e sustentáveis. Nesse contexto, a aquacultura, que envolve a criação de organismos aquáticos, emerge como uma solução promissora desempenhando um papel fundamental na colmatação dessa procura global crescente por proteínas de elevada qualidade. Desta forma, esta indústria torna-se crucial para garantir a segurança alimentar e promover o bem-estar nutricional da população mundial em constante expansão.

Tradicionalmente, a farinha de peixe é considerada a fonte de proteína padrão utilizada em dietas de muitas espécies de peixe produzidas em aquacultura, devido a um alto teor de proteínas, perfil de aminoácidos essenciais equilibrado, boa digestibilidade dos nutrientes, elevada palatabilidade e ausência de fatores antinutricionais. No entanto, devido a preocupações ambientais, ecológicas e económicas, um grande esforço tem vindo a ser feito para se transitar para uma aquacultura mais sustentável e consciente. Desse modo, proteínas vegetais são consideradas uma alternativa mais sustentável à farinha de peixe em dietas para aquacultura. Apesar disso, as proteínas vegetais apresentam algumas desvantagens, incluindo desequilíbrios nos perfis de aminoácidos e minerais essenciais, palatabilidade reduzida e a presença de fatores antinutricionais, que afetam o desempenho e crescimento dos peixes, a saúde intestinal e também a sua imunidade. Nesse sentido, estudos recentes demonstraram que a incorporação, em baixas proporções, de hidrolisados de proteínas marinhas contendo péptidos bioativos pode melhorar a qualidade nutricional de dietas com alto teor de proteínas vegetais, resultando em efeitos positivos no que concerne ao crescimento e à robustez dos peixes. Esses péptidos bioativos consistem em sequências de 2 a 20 aminoácidos que estão inativos dentro da proteína original, mas que ao serem libertados por hidrólise, tornam-se ativos e apresentam uma grande variedade de atividades bioativas potenciais, incluindo antimicrobiana e imunomoduladora. Além disso, a inclusão de hidrolisados de proteínas marinhas em formulações de dietas poderá conduzir ao aumento da valorização de matérias-primas comumente descartadas permitindo o uso sustentável e responsável desses recursos, indo de encontro a uma economia mais circular. Um exemplo disso é a pele de tintureira (*Prionace glauca*) que é um subproduto frequentemente descartado pela indústria, mas que tem um enorme potencial como matéria-prima para a produção de hidrolisados. Desse modo, neste estudo, decidiu-se avaliar os efeitos da suplementação de uma dieta comercial, contendo altos níveis de proteínas vegetais, com 3% de hidrolisado de pele de tintureira no crescimento e robustez de juvenis de robalo (*Dicentrarchus labrax*), especificamente, na resistência a episódios de stresse e a agentes potencialmente infeciosos, tal como *Photobacterium damselae* subsp. *piscicida*.

Para tal, após cerca de 13 semanas de ensaio de crescimento com a dieta controlo (CTRL) e a dieta experimental (SHARK), os peixes foram expostos a um desafio de stresse simulando uma prática comum no contexto da aquacultura, neste caso uma pesca. De facto, o robalo é particularmente suscetível ao stresse, sendo esta sensibilidade considerada um dos principais problemas da sua produção. Em situações agravadas, o stresse pode ser um fator de diminuição do sistema imunitário dos peixes e assim provocar o aparecimento de doenças. Considerando a crescente preocupação com o bem-estar dos peixes, dietas equilibradas devem fornecer não apenas os nutrientes essenciais, mas também ajudar os peixes a lidar com as situações stressantes que enfrentam. Tendo isso em consideração, e apesar de ser uma espécie robusta, o robalo é suscetível a um vasto

leque de doenças, principalmente de origem bacteriana. Mais uma vez, isto tem um impacto significativo na produção desta espécie e pode inclusive impedir a expansão da indústria. Sendo assim, também se decidiu fazer um ensaio de infeção com a bactéria responsável pela fotobacteriose. Essa doença é de difícil erradicação mesmo utilizando antibióticos, e as vacinas têm fornecido resultados variáveis contra esse patógeno. Assim, existe a necessidade de desenvolvimento de alternativas mais seguras, eficientes e económicas para a prevenção e mitigação deste tipo de doenças que afetam atualmente o setor da aquacultura do robalo.

Em relação aos parâmetros de crescimento, apenas o coeficiente de eficiência proteica (PER) foi significativamente afetado, atingindo um valor superior nos peixes alimentados com a dieta CTRL. No entanto, os demais parâmetros de crescimento não foram afetados, indicando uma boa aceitação, metabolização e utilização da dieta experimental SHARK. Na composição corporal final dos peixes, apenas as cinzas foram afetadas, sendo o seu valor mais alto no tratamento SHARK, possivelmente devido ao perfil mineral dos hidrolisados. Em relação aos metabolitos plasmáticos, observou-se a resposta usual dos peixes a um desafio de stresse, com níveis mais elevados de glicose, lactato e cortisol. No entanto, curiosamente, foi observada uma diminuição significativa nos níveis de cortisol em peixes alimentados com a dieta SHARK, evidenciando uma possível melhor utilização da energia diante das mudanças nas condições ambientais. Além disso, não existem evidências de um impacto significativamente negativo no metabolismo dos animais. Tendo em consideração os marcadores de imunidade inata, os níveis de peroxidase foram superiores no tratamento CTRL após o desafio de stresse. Porém, não há evidências de comprometimento do sistema imunológico em peixes alimentados com a dieta SHARK, pois todos os outros marcadores imunológicos não foram alterados pela dieta. Para o ensaio de infeção com a bactéria *Photobacterium damselae* subsp. *piscicida*, não foram encontradas diferenças significativas entre os dois tratamentos, resultando ambos em mortalidades cumulativas baixas.

O presente estudo mostra assim que uma pequena inclusão de hidrolisado de pele de tintureira em dietas com baixa percentagem de farinha de peixe não produz, em geral, nenhum impacto prejudicial significativo no crescimento e na robustez geral de juvenis de robalo. Além de ser um ingrediente alternativo válido à substituição de uma pequena quantidade de farinha de peixe (<25%) em termos de aceitabilidade e crescimento, parece ainda melhorar os mecanismos fisiológicos relacionados com a resposta imune, modulação do stresse e resistência a doenças. Isso pode indicar que o hidrolisado de pele de tintureira é um ingrediente seguro e funcional para ser incorporado em dietas para juvenis de robalo. Neste sentido, a valorização deste subproduto obtido a partir da indústria da pesca pode representar uma fonte de novos produtos de valor acrescentado, trabalhando no sentido do desperdício zero e assim promovendo uma economia circular e sobretudo mais sustentável.

Palavras-chave: subprodutos de pescado; hidrolisados; péptidos bioativos; dieta funcional; dieta sustentável; economia circular

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AB – Antibodies

ABW – Average body weight

ACH50 – Alternative complement pathway

ACTH – Adrenocorticotropic hormone

ANOVA – Analysis of variance

AOAC – Association of Official Agricultural Chemists

APROMAR – Asociación Empresarial de Acuicultura de España

BSSH – Blue shark skin hydrolysate

Ca^{2+} – Calcium ions

CF – Crude fat

CP – Crude protein

CRH – Corticotropin-releasing hormone

CTRL – Control diet

DGI – Daily growth index

DM – Dry matter

ELISA – Enzyme-linked immunosorbent assay

ETSA – Empresa Transformadora de Subprodutos Animais

EU – European Union

EUMOFA – European Market Observatory for Fisheries and Aquaculture Products

FAO – Food and Agriculture Organization of the United Nations

FBL – Final body length

FBW – Final body weight

FCR – Feed conversion ratio

FFA – Free fatty acids

FM – Fishmeal

HBSS – Hanks' balanced salt solution

HSD – Honestly significant difference

HSI – Hepatosomatic index

K – Condition factor

MCP – Monocalcium phosphate

Mg^{2+} – Magnesium ions

MPHs – Marine protein hydrolysates

N – Nitrogen

NRC – National Research Council

OD – Optical density

PBS – Phosphate-buffered saline

PEPT1 – Intestinal oligopeptide transporter 1

PER – Protein efficiency ratio

Pr – Proteins

RAS – Recirculating aquaculture system

SE – Standard error

SGR – Specific growth rate

SHARK – Experimental diet

VFI – Voluntary feed intake

VSI – Viscerosomatic index

WG – Weight gain

WW – Wet weight

List of Symbols

% – Percentage

‰ – Parts per thousand

°C – Degrees celsius

CFU – Colony-forming unit

cm – Centimeters

d – Days

dL – Deciliters

EU – Enzyme unit

g – Grams

***g* –** Relative centrifugal force

h – Hours

kg – Kilograms

kJ – Kilojoules

L – Liters

M – Molar

m^3 – Cubic meter

mg – Milligrams

min – Minutes

mL – Milliliters

mm – Millimeters

mM – Millimolar

mmol – Millimoles

n – Total number of individuals

ng – Nanograms

nm – Nanometers

µg – Micrograms

µL – Microliters

1. Introduction

1.1. General overview of the aquaculture sector

Aquaculture is defined as "the farming of aquatic organisms, including fish, molluscs, crustaceans, and aquatic plants, which implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc." (FAO, 1988). As one of the fastest-growing food-producing industries (Eroldoğan et al., 2022), aquaculture plays an important role in meeting the global demand for high-quality proteins, essential amino acids, vitamins, minerals, and omega-3 fatty acids, which provide numerous health benefits, contributing to a balanced diet (FAO, 2022). Aquatic foods are considered one of the most important sources of animal protein in the world, contributing in 2019 to 17% of total animal-source protein for human consumption and to 7% of all protein sources consumed (FAO, 2022). Therefore, aquaculture has become an essential part of the global food system and provides a sustainable solution to the increasing demand for high-quality protein (FAO, 2022; Velasco et al., 2023).

According to FAO (2022), in 2020 the global fisheries and aquaculture production of aquatic animals reached 178 million tonnes. From this, 88 million tonnes were produced by aquaculture, contributing to 49% of the global production of aquatic animals (Figure 1.1). Adding the production of algae to that of aquatic animals, the total fisheries and aquaculture production achieved 214 million tonnes, with aquaculture alone accounting for around 123 million tonnes. The growth of aquaculture production has been remarkable over the past few decades, while capture fisheries have stagnated since the 1990s (Waite et al., 2014).

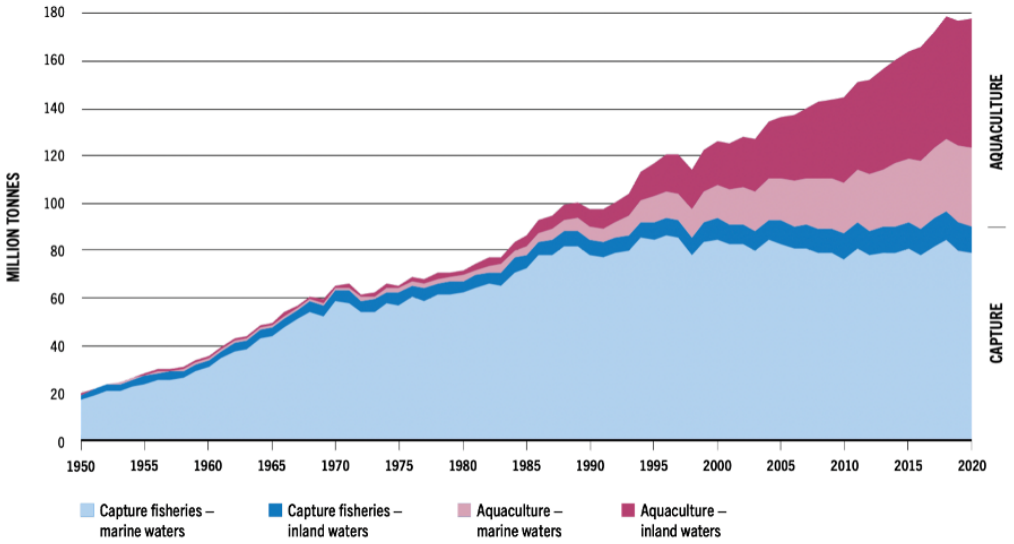

Figure 1.1 – World capture fisheries and aquaculture production (excluding algae) (FAO, 2022).

From the global fisheries and aquaculture production of aquatic animals, around 89% (158 million tonnes) were used for human consumption, while the remaining 11% (20 million tonnes) were destined for non-food uses (mostly used to produce fishmeal and fish oil). Concerning the annual per capita consumption of aquatic animal foods, it increased from 9 kg in 1961 to 20.5 kg in 2019, declining slightly to around 20.2 kg in 2020 (Figure 1.2) (FAO, 2022). This upward trend in consumption is an indication of the role played by the aquaculture industry in meeting the growing demand for food (FAO, 2022).

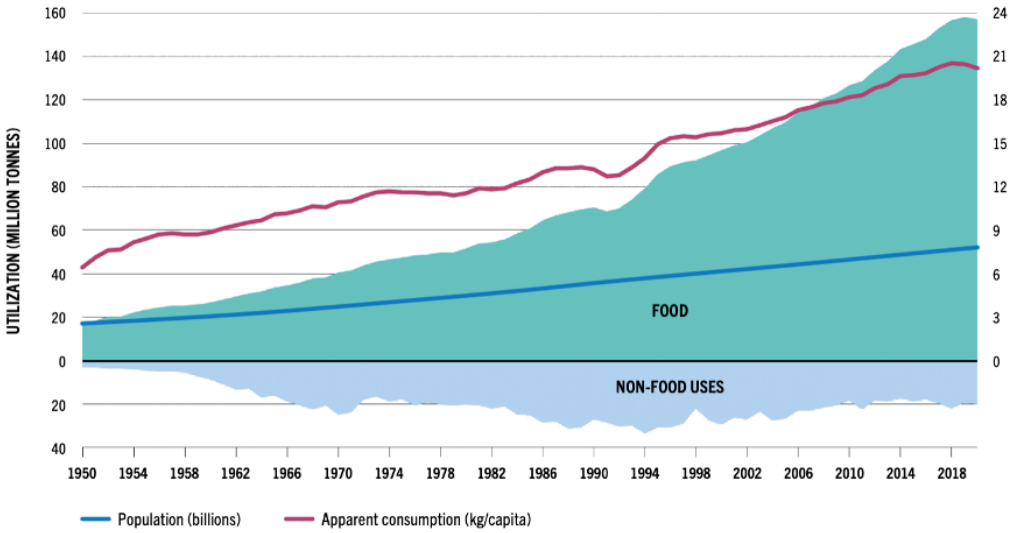

Figure 1.2 – World fisheries and aquaculture production: utilization and apparent consumption (excluding algae) (FAO, 2022).

In relation to the distribution of world aquaculture production, Asia undoubtedly leads with a share of 91.6%, followed by the Americas (3.6%), Europe (2.7%), Africa (1.9%), and Oceania (0.2%) (Figure 1.3) (FAO, 2022). Almost half (46.9%) of the entire global aquaculture harvest in 2020 consisted of finfish (57.5 million tonnes). Algae represented 28.6% (35.1 million tonnes), followed by molluscs with 14.5% (17.7 million tonnes), crustaceans with 9.2% (11.2 million tonnes), and other aquatic animals with 0.8% (1.1 million tonnes) (Figure 1.4) (FAO, 2022).

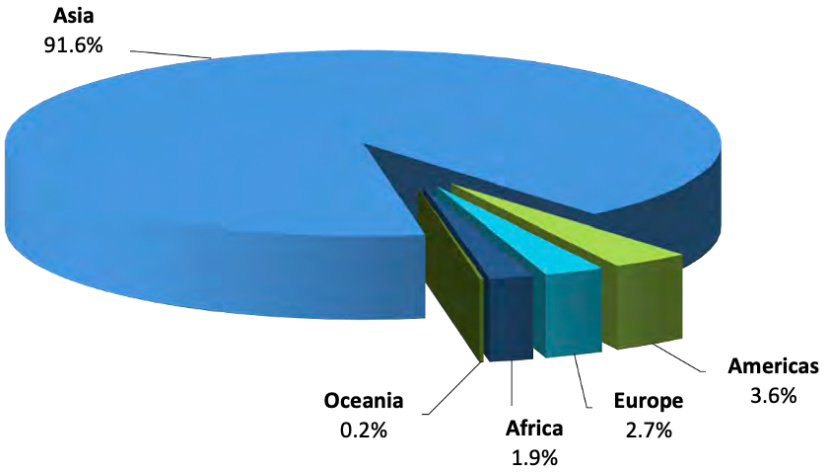

Figure 1.3 – Distribution of aquaculture production across the five continents in 2020 (adapted from APROMAR, 2022)**.**

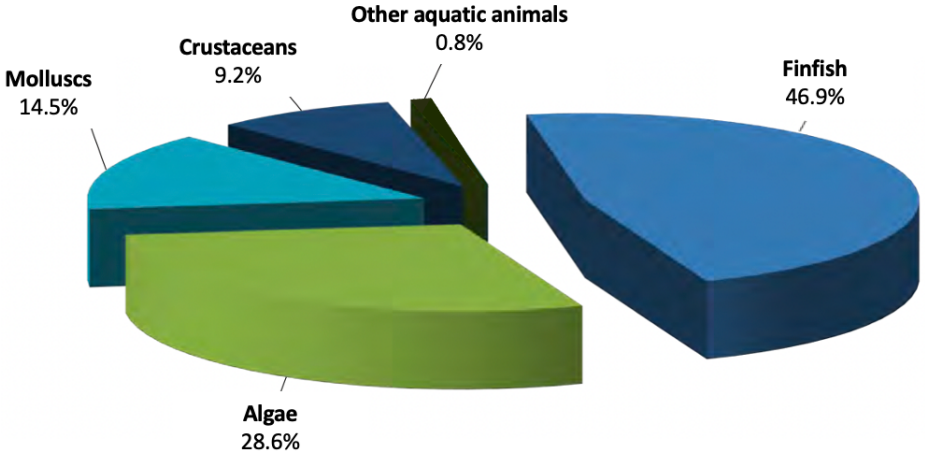

Figure 1.4 – Percentage distribution of world aquaculture production by groups in 2020 (adapted from APROMAR, 2022)**.**

In 2020, the main aquaculture species produced in the world were the Japanese kelp (*Laminaria japonica*), Eucheuma seaweeds (*Eucheuma* spp.), whiteleg shrimp (*Penaeus vannamei*), grass carp (*Ctenopharyngodon idellus*), cupped oysters (*Crassostrea* spp.), Gracilaria seaweeds (*Gracilaria* spp.), silver carp (*Hypophthalmichthys molitrix*), Nile tilapia (*Oreochromis niloticus*), Japanese carpet shell (*Ruditapes philippinarum*), and common carp (*Cyprinus carpio*) (Table 1.1) (FAO, 2022). Together, they accounted for a production of around 61 million tonnes, equivalent to almost 50% of the total aquaculture production, when including algae.

Table 1.1 – Major species (including algae) produced by aquaculture in the world in 2020 (FAO, 2022).

Species	Scientific name	Million tonnes (live weight)
Japanese kelp	*Laminaria japonica*	12.47
Eucheuma seaweeds	*Eucheuma* spp.	8.13
Whiteleg shrimp	*Penaeus vannamei*	5.81
Grass carp	*Ctenopharyngodon idellus*	5.79
Cupped oysters	*Crassostrea* spp.	5.45
Gracilaria seaweeds	*Gracilaria* spp.	5.18
Silver carp	*Hypophthalmichthys molitrix*	4.90
Nile tilapia	*Oreochromis niloticus*	4.41
Japanese carpet shell	*Ruditapes philippinarum*	4.27
Common carp	*Cyprinus carpio*	4.24

As the world's population is projected to increase to 9.7 billion in 2050 (United Nations, 2022), the demand for food will also increase by around 25-70% (Hua et al., 2019). Specifically, the demand for aquatic foods is expected to rise to an apparent per capita consumption of aquatic foods of around 22 kg by 2050 (FAO, 2022). However, due to the stagnation of wild fisheries catch, all the future increase in world fish production, to cater this increase in the human population and per capita consumption, will need to come from aquaculture (Waite et al., 2014). It is estimated that aquaculture production will increase to roughly 140 million tonnes in 2050 (excluding algae) to meet this demand (Figure 1.5) (Searchinger et al., 2019; FAO, 2022).

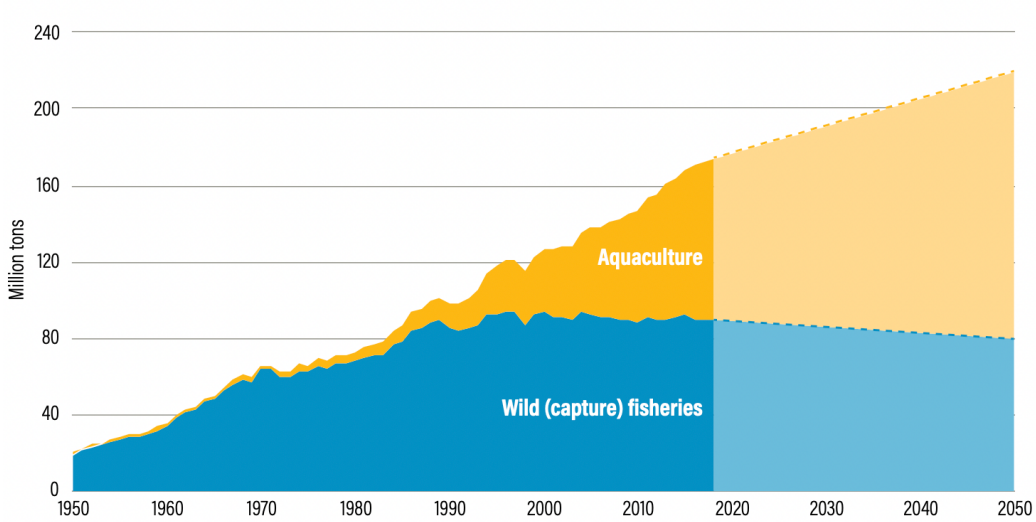

Figure 1.5 – Aquaculture production projection up to 2050 (Searchinger et al., 2019).

1.2. Protein sources used in fish feeds

Fishmeal is generally considered the gold standard dietary protein source for many fish species produced in aquaculture (Egerton et al., 2020) due to a rich protein content, well-balanced essential amino acid profile, good nutrient digestibility, and lack of anti-nutritional factors (Daniel, 2018). However, with the shift to a more sustainable-oriented aquaculture, a major effort is being made to reduce the use of fishmeal (protein source) and fish oil (lipid source) for environmental, ecological, and economic concerns (Duarte et al., 2009). In addition, prices for fishmeal and fish oil have more than doubled during the 2000s and have remained consistently higher than plant-based alternatives since 2012, due to limited supply (Figure 1.6) (Naylor et al., 2021).

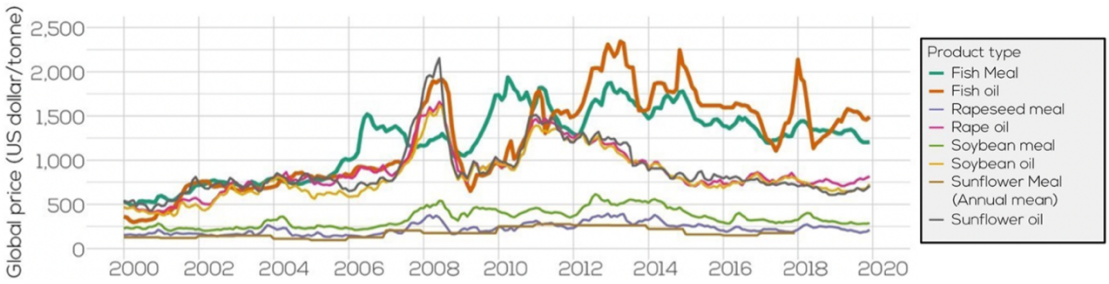

Figure 1.6 – Trend of prices of fishmeal and fish oil versus plant-based meals and oils (adapted from Naylor et al., 2021).

Because of these drawbacks, the aquaculture sector has made significant efforts to lessen its dependency on fishmeal and fish oil. Globally, the inclusion level of fishmeal in farmed fish diets has fallen significantly since 1995 and this trend is expected to

continue in the future (Figure 1.7) (Tacon and Metian, 2008; Waite et al., 2014). Numerous researchers and industry experts believe that the use of alternative protein and lipid sources will be essential for the long-term sustainability of the aquaculture sector (Naylor et al., 2021). As a result, there has been a growing interest in the use of plant-, insect-, and microbial-based protein sources in fish diets (Aragão et al., 2022).

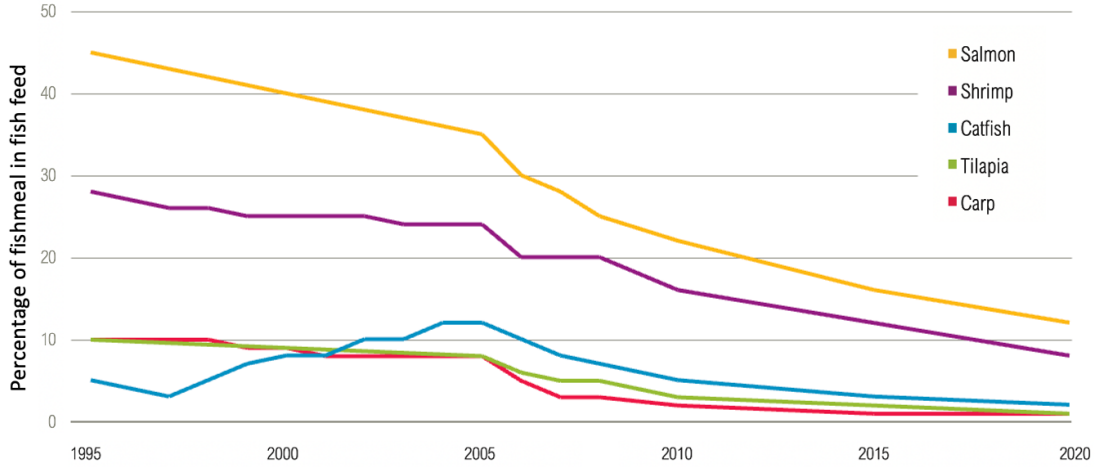

Figure 1.7 – Inclusion (%) of fishmeal in farmed fish diets (adapted from Waite et al., 2014).

While plant-based ingredients are the most common sources for replacement of fishmeal in aquafeeds (Egerton et al., 2020), they can present some drawbacks. Many plant-based sources contain anti-nutritional factors (Francis et al., 2001), amino acids imbalances, and low palatability due to high levels of non-soluble carbohydrates (Daniel, 2018) with consequent negative effects, particularly to carnivorous species, on fish growth performance, intestinal health, and immunity (Lin and Luo, 2011; Krogdahl et al., 2015; Torrecillas et al., 2017; Conde-Sieira et al., 2018; Ng et al., 2019; Xu et al., 2019; Aragão et al., 2022).

Concerning this, Robert (2014) and Costa et al. (2020) found that to overcome the challenge of replacing increasing levels of fishmeal with plant sources, low levels of marine protein hydrolysates can be incorporated into aquafeeds. This results in increased palatability, reduction of amino acids imbalances, and increase of adequate bioavailable nutrients (Espe et al., 2007; Egerton et al., 2020), reducing the negative effects associated with the incorporation of high levels of plant-based ingredients in fish diets (Costa et al., 2020; Siddik et al., 2021a).

1.3. Marine protein hydrolysates in fish nutrition

Marine protein hydrolysates (MPHs) can be obtained from either autolysis, bacterial fermentation, chemical hydrolysis (acid and alkaline), or enzymatic hydrolysis (Siddik et al., 2021a). They are composed of marine proteins broken down into single amino acids, peptides, and oligopeptides (Kristinsson and Rasco, 2000). Protein hydrolysates, the result of the beforementioned methods, are rich in bioactive peptides (Idowu et al., 2020) with properties beyond their nutritional value (Resende et al., 2022), making them potential nutraceuticals and functional food additives (Cardoso and Nunes, 2013).

Enzymatic hydrolysis has been demonstrated to be an efficient method for recovering bioactive peptides from waste material (Thiansilakul et al., 2007) and has the advantage of working with a shorter reaction time, which is useful for targeting certain peptide bonds and amino acids that have optimal activity at specific conditions; moreover, it does not produce any residual organic solvents and toxic chemicals in the end products (Najafian and Babji, 2012). However, its biggest disadvantage is the high cost of the process (Hou et al., 2017). Various proteolytic enzymes such as alcalase, neutrase, papain, pepsin, and trypsin, can be used for this process (Kristinsson and Rasco, 2000). Among them, alcalase produced by a Gram-positive bacteria (*Bacillus licheniformis*) has been described as one of the best enzymes that can be used for producing protein hydrolysates (Guérard et al., 2001), owing to its high extraction ability under mild conditions and ability to produce protein hydrolysates with small-sized peptides in a relatively short period (Kristinsson and Rasco, 2000).

Marine protein hydrolysates can be produced from fish processing by-products, fisheries bycatch, and low-value pelagic species that are not currently consumed directly by humans (Kristinsson et al., 2007; Egerton et al., 2018). Among 20-80% of a fish is considered waste by the fish processing industry, depending on several parameters such as the fish type and the processing specifications (Caldeira et al., 2018; Siddiqui et al., 2023), and usually includes the head (9-12% of the whole-body weight), viscera (12-18%), skin and fins (1-3%), bones (9-15%), muscle trimmings (15-20%) and scales (5%) (Figure 1.8) (Guérard, 2009). In Europe, in 2016, there were around 0.6 million tonnes of unutilized seafood by-products (Jackson and Newton, 2016).

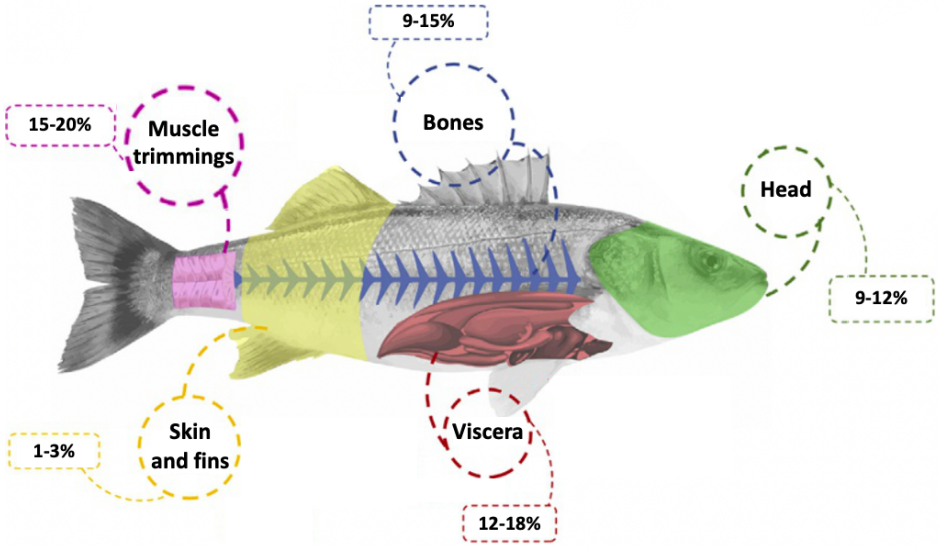

Figure 1.8 – Average proportion (%) of fish by-products (modified from Al Khawli et al., 2020).

The production of marine protein hydrolysates is influenced by many factors, including the composition of the raw material, the type of enzyme used, hydrolysis conditions, and the degree of hydrolysis (Benjakul et al., 2014; Ramakrishnan et al., 2023). The production process comprises three phases: i) a pretreatment phase which involves the formation of a homogenized water-by-product mixture, with the lowest fat content possible and without other undesirable components, for subsequent hydrolysis; ii) a hydrolysis phase in which an enzyme is added to resulting on the cleavage of the peptide bonds to obtain free amino acids and low molecular weight peptides; iii) a recovery phase where marine protein hydrolysates, with enhanced functional and bioactive properties, are obtained (Figure 1.9) (He et al., 2013; Nghia et al., 2020). In general, the yield in the production of protein hydrolysates is low, since only the soluble fraction (supernatant), which contains the products of interest, is used in the drying stage (Khantaphant et al., 2011).

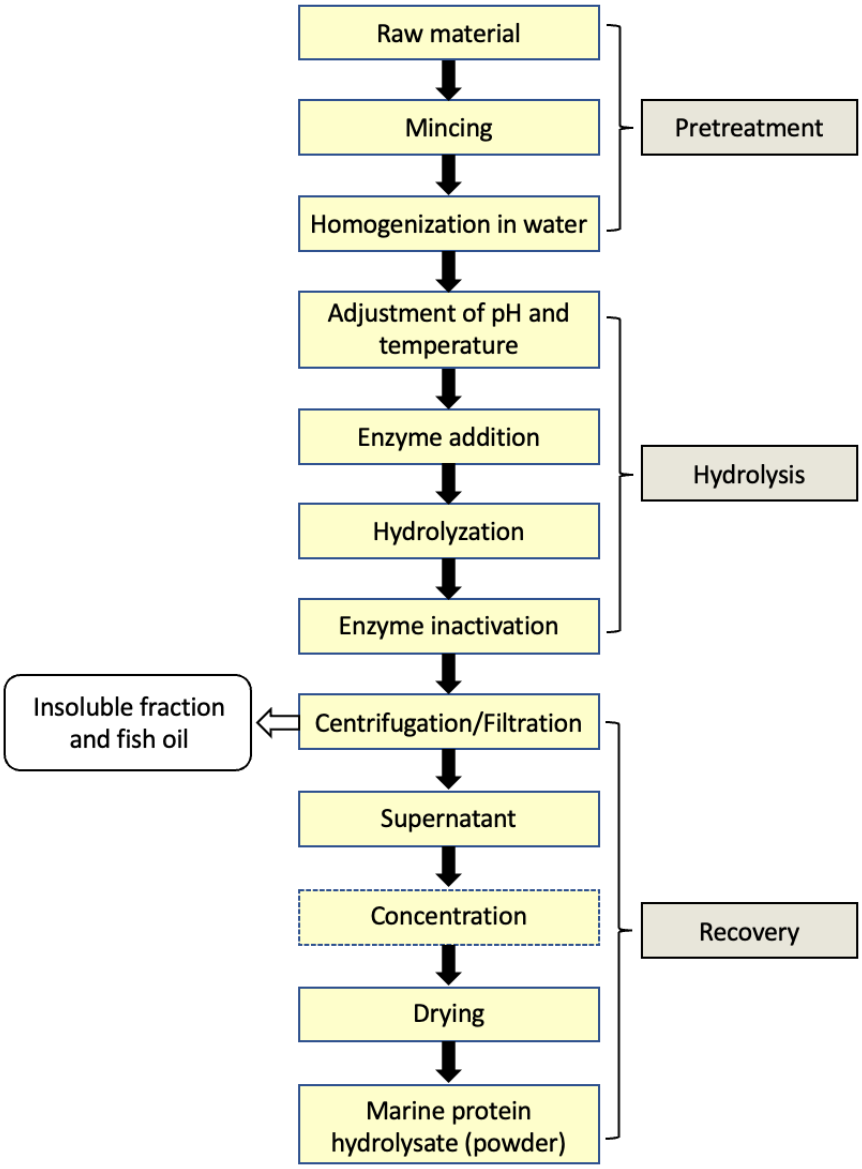

Figure 1.9 – Production scheme of marine protein hydrolysates (based on Batista et al., 2006; He et al., 2013; Villamil et al., 2017). A concentration step (optional) in the recovery phase can be used to lower the water content reducing the final energy requirements before the drying process and minimizing the environmental impacts of the overall production process (Petrova et al., 2018; Sherman, 2022).

The variable composition of processing by-products from batch to batch presents a significant challenge in the production of MPHs, as it results in variable nutritional composition and makes it difficult to obtain a consistent end-product. Furthermore, the production of bioactive peptides from fish waste using enzymatic hydrolysis has been generally reported only from small-scale or controlled laboratory systems, due to the high cost of the enzymes used. So, more studies are needed to assess its economic feasibility under industrial production conditions (Siddik et al., 2021a).

1.3.1. Bioactive peptides present in MPHs

Bioactive peptides consist of sequences of 2 to 20 amino acids that are inactive within the original protein, but upon release by hydrolysis, they become active and present several physiological functions (Batista, 2013; Marti-Quijal et al., 2020).

The information obtained so far suggests that the functional properties of protein hydrolysates result from their amino acid and peptide composition (Espe et al., 1999), as well as peptide molecular weight (Liaset et al., 2000). Low molecular weight peptides are more bioactive and potent than large molecular weight peptides because they are more easily absorbed by the gastrointestinal tract (Gu et al., 2011; Chi et al., 2015; Heffernan et al., 2021). Due to their small size, fish will spend less energy absorbing them, and that extra energy can be used for other physiological functions, such as growth (Andrade and Boscolo, 2019). Fish-derived peptides have a diverse range of bioactive activities including antioxidant, antimicrobial and immunomodulatory (Martínez-Alvarez et al., 2015; Hou et al., 2017; Ishak and Sarbon, 2018; Zamora-Sillero et al., 2018).

1.3.2. Incorporation level of MPHs on fish diets

Marine protein hydrolysates in aquafeeds have been reported to improve feed intake, feed utilization, and growth of fish (Refstie et al., 2004; Aksnes et al., 2006; Zheng et al., 2012,2013; Khosravi et al., 2015; Gunathilaka et al., 2020), as well as to promote the immune system (Liang et al., 2006; Kotzamanis et al., 2007; Ovissipour et al., 2014) and disease resistance (Siddik et al., 2019; Chaklader et al., 2020). Similar effects were also reported on shrimp (Seguin et al., 2018; Herault et al., 2020). However, those effects vary depending on the raw material from which the protein hydrolysates were obtained, the processing method, and the incorporation level in the diets.

Low incorporation levels (1 to 5%) of MPHs seem to improve growth and immune response in several fish species, such as red seabream (*Pagrus major*) (Khosravi et al., 2015), Asian seabass (*Lates calcarifer*) (Chotikachinda et al., 2013; Novriadi et al., 2015), striped catfish (*Pangasianodon hypophthalmus*) (Soller et al., 2021), tilapia (*Oreochromis* sp.) (Herault et al., 2022), pabda (*Ompok pabda*) (Suma et al., 2023), and European seabass (*Dicentrarchus labrax*) (Fournier et al., 2011; Gisbert et al., 2018; Leduc et al., 2018a,b). Low incorporation levels also shown to increase fish resistance to

pathogens such as *Vibrio pelagius* on European seabass (Gisbert et al., 2018), *Vibrio harveyi* on Asian seabass (Siddik et al., 2021b), *Edwardsiella tarda* on red seabream (Bui et al., 2014), and *Aeromonas hydrophyla* on Nile tilapia (Herault et al., 2012).

Moderate incorporation levels (5 to 10%) also seem to improve growth performance, immunity, and disease resistance of some fish, such as large yellow croaker (*Pseudosciaena crocea*) (Tang et al., 2008), Asian seabass (Siddik et al., 2018), and South American catfish (*Rhamdia quelen*) (Ha et al., 2019). Also, in a study by Kotzamanis et al. (2007), the inclusion of 10% of a protein hydrolysate improved intestinal development, growth, immunological status, and survival of European seabass larvae challenged with *Vibrio anguillarum*.

In contrast, high dietary levels (10 to >20%) are normally associated with higher production costs and impairment of fish growth performance (Martínez-Alvarez et al., 2015; Xu et al., 2016; Pham et al., 2022). The negative impacts of high levels of marine hydrolysates incorporation on fish performance are probably due to an excessive amount of free amino acids and short-chain peptides in the intestine, which could induce the saturation and competition of transporters mechanisms (Ospina-Salazar et al., 2016; Siddik et al., 2018).

Therefore, through the assessment of various studies using diets with different levels of inclusion of MPHs, there seems to be no specific optimal inclusion level. However, inclusion levels ranging from 3-10% have been found to be acceptable and well-documented. In this study, a conservative value of 3% inclusion level was chosen, which has shown overall more positive effects on different fish species. This approach allows for a safer and more reliable use of MPHs in fish diets, while still providing the benefits associated with their incorporation.

1.4. Blue shark skin waste

The blue shark (*Prionace glauca*) (Figure 1.10) is the most widely distributed and fished shark species in the world, normally caught as an accessory species. As a carnivorous species, it feeds on small prey such as bony fishes, cephalopods (mainly squids), and small sharks. This species prefers water temperatures from 7 to 16 °C but can tolerate water up to 21 °C or even higher (FAO, 2023a).

The capture of this species rose considerably in the late 1990s for the consumption of shark meat and the production of nutritional supplements and fin soup (Batista et al., 2022). This has resulted in significant amounts of shark skin waste, which is commonly discarded due to the difficulty of converting the skin into fishmeal (Mastandrea, 2021).

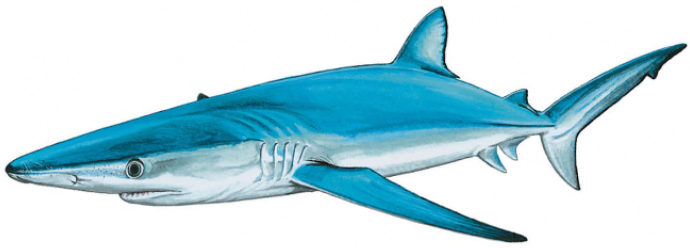

Figure 1.10 – Blue shark (Shark Research Institute, 2023).

The blue shark is an oceanic circumglobal species, which can be found in all the major oceans of the world, from tropical to temperate waters (Figure 1.11) (FAO, 2023a).

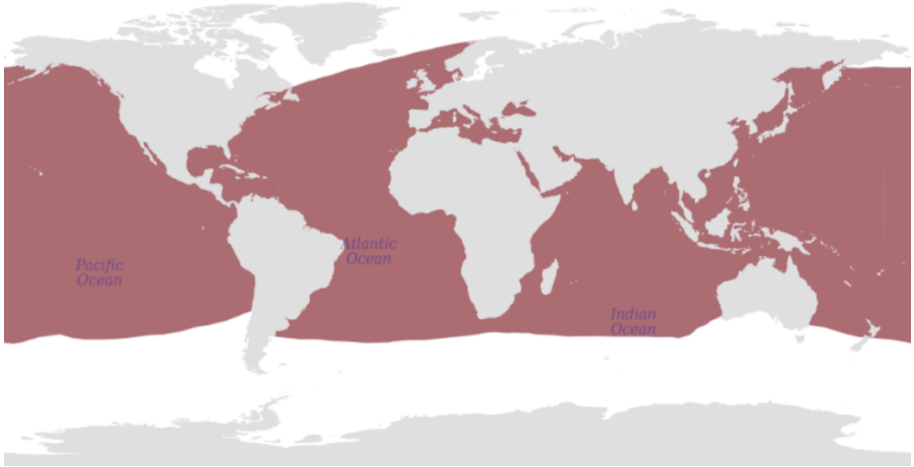

Figure 1.11 – Distribution map of blue shark (FAO, 2023a).

In 2020, the global capture of blue sharks was approximately 93 thousand tonnes (FAO, 2023a). However, the capture of this species peaked in 2013 and has been decreasing, which may indicate a population decline (Figure 1.12) (Okes and Sant, 2019).

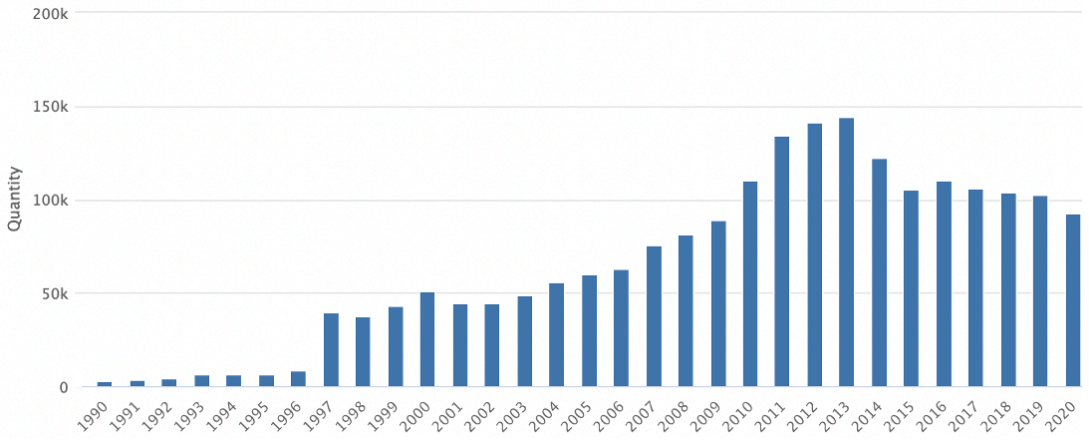

Figure 1.12 – World capture production of blue shark, from 1990 to 2020 (adapted from FAO, 2023a).

Important to note that in 2019 a landing obligation by the European Commission (under Article 15 of the Common Fisheries Policy) was implemented, forcing all fishing vessels to land previously discarded species that are subjected to quota or have a minimum legal size, as well as underutilized commercial species (European Commission, 2013). Given the increased interest in the circular economy by the European Union (EU) (European Commission, 2020), the utilization of underutilized or formerly discarded marine material might offer a sustainable strategy for the production of high-added value compounds (Coppola et al., 2021). To ensure the long-term viability of the seafood industry, it is crucial to use the whole animal as efficiently as possible (Malcorps et al., 2020).

1.5. European seabass

The European seabass (Figure 1.13) is one of the most popular and successful species in the European aquaculture sector nowadays, especially in the Mediterranean (Sánchez et al., 2022), being in 2020 the third most important aquaculture species in Europe in terms of value (EUMOFA, 2022). This species is carnivorous (feeding range includes small fish, prawns, crabs, and cuttlefish), eurythermal (5-28 °C), euryhaline (3 ‰ to full strength seawater), and gonochoristic with a high fecundity spawning once per year, from December to March in the Mediterranean population and up to June in the Atlantic populations (Haffray et al., 2007; FAO, 2023b).

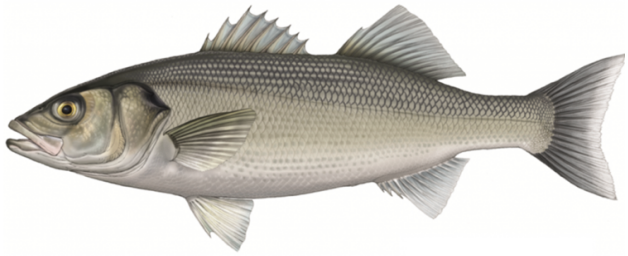

Figure 1.13 – European seabass (Seafish, 2023).

The European seabass is geographically distributed in the North Atlantic, from Norway and the British Isles southward to Morocco and the Canary Islands. It is also present in the Mediterranean and Black Seas, and southward to Senegal (Figure 1.14) (FAO, 2023c).

Figure 1.14 – Distribution map of European seabass (FAO, 2023c).

In 2020, the global aquaculture production of European seabass was around 276 thousand tonnes (FAO, 2023c). The production of this species has been constantly increasing since 1990 (Figure 1.15).

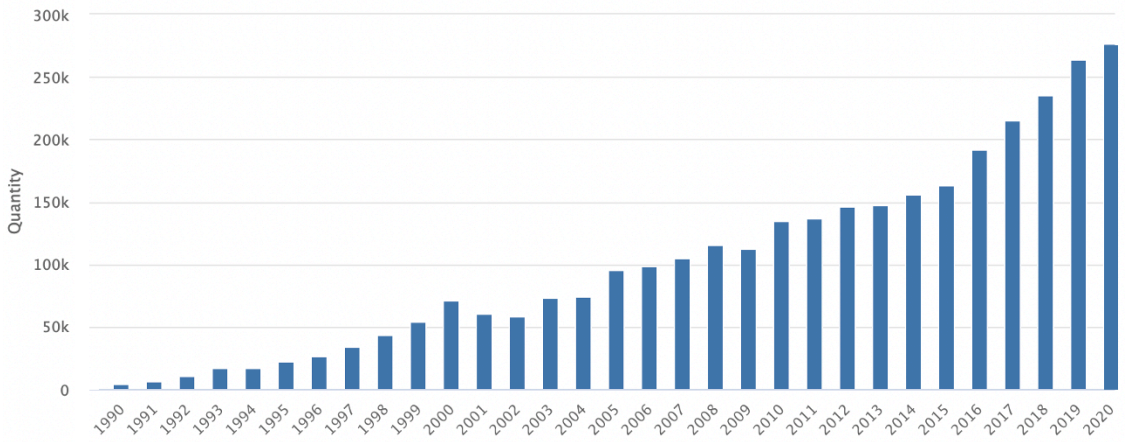

Figure 1.15 – World aquaculture production of European seabass, from 1990 to 2020 (adapted from FAO, 2023c).

1.5.1. Main diseases in European seabass – Photobacteriosis

Despite being a sturdy species, the European seabass is susceptible to a wide range of diseases, particularly caused by bacteria, under rearing conditions (Table 1.2). These outbreaks have a significant impact on commercial production and may prevent the expansion of the industry in some countries (FAO, 2023b). Among the most important diseases, tenacibaculosis, vibriosis, and photobacteriosis are the most prominent in European seabass (Spinos et al., 2017; Muniesa et al., 2020).

Table 1.2 – Major bacterial pathogens of economically important fish (seabass is outlined for easier identification) (adapted from Sudheesh et al., 2012)

Causative agent/species	Disease	Main host fish
Gram-negatives		
Vibrio anguillarum	Vibriosis	Salmonids, turbot, sea bass, striped bass, eel, ayu, cod, and red sea bream
Aliivibrio salmonicida (formerly *Vibrio salmonicida*)	Vibriosis	Atlantic salmon, cod
Vibrio vulnificus	Vibriosis	Eels, tilapia
Vibrio ordalii	Vibriosis	Salmonids
Vibrio carchariae (syn.: *Vibrio harveyi*)	Vibriosis, infectious gastroenteritis	Shark, abalone, red drum, sea bream, sea bass, cobia, and flounder
Moritella viscosa (formerly *Vibrio viscosus*)	Winter ulcer	Atlantic salmon
Photobacterium damselae subsp. piscicida (formerly *Pasteurella piscicida*)	Photobacteriosis (pasteurellosis)	Sea bream, sea bass, sole, striped bass, and yellowtail
Pasteurella skyensis	Pasteurellosis	Salmonids and turbot
Tenacibaculum maritimum (formerly *Flexibacter maritimus*)	Flexibacteriosis	Turbot, salmonids, sole, sea bass, gilthead sea bream, red sea bream, and flounder
Flavobacterium psychrophilum	Coldwater disease	Salmonids, carp, eel, tench, perch, ayu
Flavobacterium branchiophila	Bacterial gill disease	A broad range of cultured cold water and warm water salmonid and nonsalmonid fishes
Flavobacterium columnare	Columnaris disease	cyprinids, salmonids, silurids, eel, and sturgeon
Pseudomonas anguilliseptica	Pseudomonadiasis, winter disease	Sea bream, eel, turbot, and ayu
Aeromonas salmonicida	Furunculosis	salmon, trout, goldfish, koi and a variety of other fish species
Aeromonas hydrophila *Aeromonas veronii* Biovar Sobria *Aeromonas sobria* Biovar Sobria (Motile aeromonads)	Motile aeromonas septicemia (MAS), hemorrhagic septicemia, ulcer disease or red-sore disease, and epizootic ulcerative syndrome (EUS)	A wide variety of salmonid and nonsalmonid fish, sturgeon, tilapia, catfish, striped bass, and eel
Edwardsiella ictaluri	Enteric septicemia	Catfish and tilapia
Edwardsiella tarda	Edwardsiellosis	Salmon, carps, tilapia, catfish, striped bass, flounder, and yellowtail
Yersinia ruckeri	Enteric redmouth	Salmonids, eel, minnows, sturgeon, and crustaceans
Piscirickettsia salmonis	Piscirickettsiosis	Salmonids
Gram-positives		
Lactococcus garvieae (formerly *Enterococcus seriolicida*)	Streptococcosis or lactococcosis	Yellowtail and eel
Streptococcus iniae	Streptococcosis	Yellowtail, flounder, sea bass, and barramundi
Streptococcus parauberis	Streptococcosis	Turbot
Streptococcus phocae	Streptococcosis	Atlantic salmon
Renibacterium salmoninarum	Bacterial kidney disease	Salmonids
Mycobacterium marinum	Mycobacteriosis	Sea bass, turbot, and Atlantic salmon

Photobacterium damselae subsp. *piscicida*, a Gram-negative bacteria, results in serious losses among cultured fish species in Europe including gilthead seabream (*Sparus aurata*), red porgy (*Pagrus pagrus*), red seabream, European seabass, meagre (*Argyrosomus regius*), and sole (*Solea* spp.). Under aquaculture conditions, the gilthead seabream and European seabass suffer most of the financial losses (Toranzo et al., 1991; Baptista et al., 1996; Bakopoulos et al., 1997; Zorilla et al., 1999). During acute cases of photobacteriosis, fish exhibit only a few pathological signs, being convulsive erratic swimming often the only clinical sign. Shortly after, anorexia, lethargy, darkening and ulceration of the skin start to occur. As the disease progresses the gills become pale with

excessive mucous secretions. Inflammation of the lip, opercula skin, and lower jaw occurs and necrotic skin patches on the body flanks, dorsal area, and tail become common. The fins may become eroded, and the liver becomes inflamed and congested. The spleen becomes enlarged, the kidney pale and the intestine becomes filled with fluid and some whitish mucous clots start to appear. In the more chronic form of the disease, pseudotuberculosis develops mainly in the spleen and/or kidney parenchyma (Varvarigos, 2020).

Antibiotics are frequently used as the first line of defense for treatment against pathogenic bacteria. A wide range of antibiotics have been developed to restrict the development of these microorganisms. Unfortunately, unregulated, and excessive usage of these chemicals has resulted in antibiotic resistance (Nirmal et al., 2022). In addition to that, and as stated by Andreoni and Magnani (2014), photobacteriosis is hard to eradicate with antibiotic treatments. Because of the numerous problems and impacts of using antibiotics, at the beginning of 2022, the EU prohibited all forms of routine antibiotic use in farming (More, 2020; Nunan, 2022).

As an alternative, research has been focused on the development of vaccines to prevent bacterial diseases and progressively reduce the use of antibiotics in aquaculture (Håstein et al., 2005). However, many common bacterial diseases in fish farms currently lack effective vaccination strategies (Gudding and Van Muiswinkel, 2013). According to Spinos et al. (2017), commercial vaccines against *Photobacterium damselae* subsp. *piscicida* have been available for quite some time. However, these commercial formulations have provided variable results against the pathogen responsible for photobacteriosis. Additionally, another problem of vaccination is the need to handle the animals which causes unnecessary additional stress (Sommerset et al., 2005).

Because of that, there is a need for the development of more safe, efficient, and cost-effective alternatives for the prevention and mitigation of numerous diseases that affect nowadays the aquaculture sector (Siddik et al., 2021a). In this regard, MPHs appear to be a good alternative (Herault et al., 2012; Novriadi et al., 2015). By using those compounds, we are shifting towards more sustainable and natural prevention infectious disease strategies (Reverter et al., 2020).

1.5.2. Stress response in fish

Stress is defined as "a condition in which the dynamic equilibrium of the organism, called homeostasis, is threatened or disturbed as a result of the actions of intrinsic or extrinsic stimuli that act as stressors" (Wendelaar Bonga, 1997). Stress triggers physiological and behavioral responses that can impair body functions, growth, and resistance to diseases, and can eventually lead to death (Mateus et al., 2017).

Unlike wild fish, farmed fish are exposed at all stages of the production cycle to environmental (temperature, salinity, ammonia, dissolved oxygen, pH, chemicals, and pathogens), physical (cleaning, grading, handling, crowding, confinement, feeding, and vaccination), and social (competition, crowding, and aggressiveness) stressors (Pavlidis and Mylonas, 2011; Tort, 2011; Braithwaite and Ebbesson, 2014). The intensity and duration of physiological responses, which are largely influenced by the nature of the stressor (acute or chronic), determine the outcome of the stress response (Davis, 2006). Acute stressors are characterized by high severity, short duration, and abrupt onset of the stress response, which is based on the fight or flight reaction, ensuring survival. Chronic stressors induce a less intense and slower onset stress response but have a higher energetic cost because of their duration and can lead to various problems, compromising survival (Martínez-Porchas et al., 2009; Tort, 2011; Ellis et al., 2012).

The stress response in fish can be divided into primary, secondary, and tertiary (Wendelaar Bonga, 1997; Barton, 2002; Sadoul et al., 2021). The primary response initiates rapidly with the activation of the adrenergic system, which causes an increase in plasma adrenaline and noradrenaline (catecholamines) and is followed by the activation of the hypothalamus–pituitary–interrenal axis and an increase in plasma cortisol. The secondary response encompasses physiological modifications and includes metabolic, hydromineral, and immunological changes. The tertiary response describes whole animal changes and is associated with a reduction in growth, resistance to disease, reproduction, behavior, and ultimately survival (Figure 1.16) (Wendelaar Bonga, 1997; Miller and O'Callaghan, 2002; Pottinger, 2008; Ellis et al., 2012).

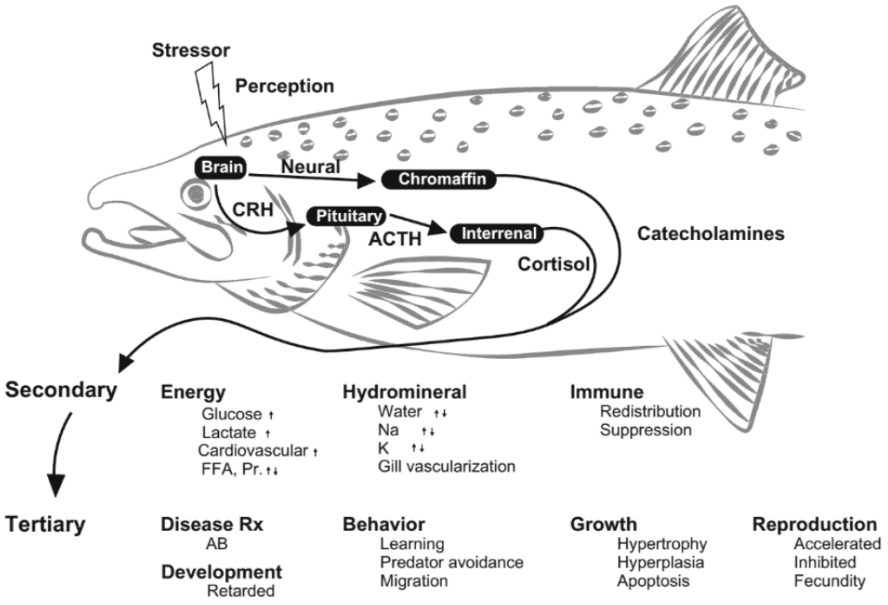

Figure 1.16 – Responses of fish to a stressor (Schreck and Tort, 2016). CRH – Corticotropin-releasing hormone; ACTH – Adrenocorticotropic hormone; FFA – Free fatty acids; Pr – Proteins; AB – Antibodies.

European seabass, the model used in the present study, is susceptible to stress, showing high levels of cortisol following handling and confinement, and this sensitivity to stress is considered one of the major issues slowing down European seabass aquaculture (Samaras et al., 2018). Considering the growing concern with fish welfare, it has become clear that well-balanced diets must not only provide the essential nutrients but also assist fish in coping with the stressful situations they face on aquaculture farms (Ashley, 2007; Machado et al., 2019). Therefore, an emergent strategy for improving fish welfare is the supplementation of aquafeeds with MPHs (Siddik et al., 2021a).

1.6. Objectives

In this work, it was hypothesized that dietary inclusion of bioactive peptides present in the blue shark skin hydrolysate could improve European seabass growth and robustness.

1.6.1. General

The general objective of this work is to investigate the effects of supplementing a highly vegetable-based commercial diet with 3% blue shark skin hydrolysate on the growth, modulation of the stress response, and increase in disease resistance of juvenile European seabass.

1.6.2. Specific

The present study has the following specific objectives: i) Provide a comprehensive study on the effects of blue shark skin hydrolysate on growth performance and overall nutritional status of juvenile European seabass; ii) Study the effects of the bioactive potential of hydrolysates obtained from shark skin to improve the response to stress generated by fish handling routines in aquaculture farms; iii) Explore the potential benefits of blue shark skin hydrolysate to improve resistance to photobacteriosis, one of the most problematic diseases in European seabass production.

2. Material and Methods

2.1. Marine hydrolysate

The hydrolysate used in the present study was obtained from blue shark caught in the Northeast Atlantic Ocean. The blue shark skin by-products were generated by Brasmar - Trade Food, S.A. (Porto, Portugal), from the production of fillets for human consumption, and were frozen, processed in an industrial grinder, and stored appropriately for further processing. Thereafter, the blue shark skin hydrolysate (BSSH) was produced by ETSA - SGPS, S.A. (Empresa Transformadora de Subprodutos Animais; Lisboa, Portugal) under industrial confidential conditions, using enzymatic hydrolysis with alcalase. The proximate composition of the hydrolysate is present in Table 2.1.

Table 2.1 – Proximate composition of the marine hydrolysate used in this study.

	Ingredient
	BSSH [a]
Proximate composition (% DM)	
Dry matter (DM, %)	97.37
Ash	5.13
Crude protein	87.87
Crude fat	0.34
Gross energy (kJ g^{-1} DM)	18.95

[a] Blue shark skin hydrolysate.

2.2. Experimental diets

Two isoproteic (52.9% dry matter, DM), isoenergetic (22.7 kJ g^{-1} DM), and isolipidic (17.8% DM) diets were formulated: a control diet (CTRL), consisting of a commercial-like diet containing 69% vegetable-content and 12.5% of fishmeal, and an experimental diet (SHARK) obtained by adding 3% of blue shark skin hydrolysate, at the expense of fishmeal (Table 2.2). The experimental diets (2 mm of pellet size) were formulated and produced by SPAROS Lda. (Olhão, Portugal), in conformity with European seabass nutrient requirements (NRC, 2011), and then stored at 4 °C.

Table 2.2 – Ingredients and proximate composition of the diets used in this work.

	Diets	
	CTRL	**SHARK**
Ingredients (%)		
Fishmeal [a]	12.5	8.5
BSSH [b]	0	3
Soy protein concentrate [c]	25	25
Wheat gluten [d]	14.4	14.4
Corn gluten meal [e]	10	10
Soybean meal [f]	11	11
Wheat meal [g]	8.98	9.18
Fish oil [h]	7.4	7.8
Rapeseed oil [i]	6.8	6.8
Vitamin and mineral premix [j]	1	1
Choline chloride 50% silica	0.2	0.2
Antioxidant powder [k]	0.2	0.2
Monocalcium phosphate	2.5	2.9
Proximate Composition (% DM)		
Dry matter (DM, %)	95.29	94.46
Ash	6.90	7.01
Crude protein	52.52	53.21
Crude fat	17.83	17.66
Gross energy (kJ g^{-1} DM)	22.44	22.87

[a] Fishmeal NORVIK LT 70: 72% crude protein (CP), 7% crude fat (CF) (Sopropêche, Wimille, France). [b] Blue shark skin hydrolysate. [c] Soycomil-P: 62% CP, 0.7% CF (ADM Animal Nutrition, Amsterdam, Netherlands). [d] Wheat gluten: 80% CP, 7% CF (Roquette Frères, Lestrem, France). [e] Corn gluten meal: 61% CP, 6% CF (COPAM, São João da Talha, Portugal). [f] Dehulled solvent extracted soybean meal: 48% CP, 2% CF (Cargill, Barcelona, Spain). [g] Wheat meal: 11% CP, 2% CF (Casa Lanchinha Lda., Alhos Vedros, Portugal). [h] Sardine oil (Sopropêche, Wimille, France). [i] Rapeseed oil (Henry Lamotte Oils GmbH, Bremen, Germany). [j] Vitamin and mineral premix: WISIUM MIX AQUA 1.5% (ADM Portugal S.A., Sepins, Portugal). [k] Antioxidant: VERDILOX (Kemin Europe NV, Herentals, Belgium).

2.3. Growth trial

European seabass juveniles (n=300) were obtained from a commercial fish farm (Acuinuga, S.L., Spain). They were transported to the CIIMAR facilities (University of Porto, Portugal) and acclimated for 15 days to the new husbandry and water conditions in a 2 m^3 square fiberglass tank, in a recirculating aquaculture system (RAS). During this period, fish were hand-fed one time per day (1% body weight) with a commercial diet (AQUASOJA, Portugal; 50% crude protein and 20% crude fat as DM basis). Before the onset of the trial, all fish were fasted for a 24 h period and afterwards slightly anesthetized using 2-phenoxyethanol (Sigma-Aldrich, MO, USA) at a concentration of 60 μL L^{-1}, and individually weighed (12.6 ± 0.11 g) and measured (10.7 ± 0.04 cm, total length); then they were distributed into 6 homogeneous groups (50 fish $tank^{-1}$; density of 3.9 kg m^{-3}). Groups were kept in 6 fiberglass tanks of 160 L, part of a RAS system. Each diet was randomly allocated to triplicate tanks. Each tank was provided with seawater (33 ‰), mechanically filtered, heated (22 °C), and oxygenated (> 90% saturation), at a flow rate of 10 L min^{-1}, under an artificial photoperiod of 12 h light : 12 h dark. Physical and chemical water parameters (temperature, salinity, oxygen saturation, redox potential, pH, and concentration of nitrogenous compounds) were daily monitored during the trial and maintained at optimal levels for this species: $NH_4^+ \leq 0.05$ mg L^{-1}, $NO_2^- \leq 0.5$ mg L^{-1}, $NO_3^- \leq 5$ mg L^{-1}, and pH 7.5-8.5 (Kır et al., 2019). Fish were fed three times a day by automatic feeders until apparent visual satiety (*ad libitum*), for a period of around 13 weeks (89 days). Fish were bulk weighed in an intermediate sampling, after 6 weeks, to monitor weight gain and feed consumption.

2.4. Stress challenge

At the end of the growth trial, 5 fish per tank (15 fish per treatment) were exposed to a stressful challenge, simulating a fishing operation: confinement stress for 5 min at a density of 100 kg m^{-3} and air exposure for 1 min. Afterwards, fish were left for 1 h in a tank with aeration, before sampling of blood and tissues.

2.5. Sampling procedures

Following the growth trial, all fish were fasted for 24 h before sampling. Initially, 5 fish per tank (15 fish per treatment) were given a mild anesthesia dose of 60 μL L^{-1} of 2-phenoxyethanol and then weighed and measured individually. Sequentially, one fish at a time was heavily anesthetized using 500 μL L^{-1} of 2-phenoxyethanol to collect blood from their caudal vein. Simultaneously, another set of 5 fish per tank (15 fish per treatment), which had been subjected to a stressful situation, was given a sedative dose of 60 μL L^{-1} of 2-phenoxyethanol and then individually weighed and measured. These fish were then heavily anesthetized using 500 μL L^{-1} of 2-phenoxyethanol, and blood was collected from their caudal vein. Blood from both groups of fish was collected using heparinized syringes and then centrifuged at 5000 × *g* for 10 min at 4 °C. The obtained plasma was used for evaluation of the stress, nutritional and immunological status. Finally, those fish were sacrificed by a cut through their spinal cord, and the intestines and livers were collected and weighed to determine the viscerosomatic (VSI) and hepatosomatic (HSI) indices, respectively. The remaining fish were anesthetized (60 μL L^{-1} of 2-phenoxyethanol) and individually weighed and measured for evaluation of growth performance and feed consumption.

For the whole-body composition analysis, 20 fish from the initial fish stock (before the growth trial started) and 5 fish per tank (15 fish per treatment) at the end of the growth trial were collected, sacrificed with an anesthetic overdose (1 mL L^{-1} of 2-phenoxyethanol), and stored at -80 °C.

2.6. Infectious challenge

Additionally, after the growth trial, 15 fish from each tank (45 fish per treatment) were transferred to a new rearing system with 120 L tanks and the following conditions: temperature – 24 ºC, salinity – 35 ‰, photoperiod – 12 h light : 12 h dark, and strong aeration. The bacterium used was *Photobacterium damselae* subsp. *piscicida* (strain MT1415), kindly provided by Andrew C. Barnes (Marine laboratory, UK). This bacterial species was cultured on Erlenmeyer flasks containing 50 mL of tryptic soy broth (TSB) supplemented with 1.5% of NaCl (Difco Laboratories, USA) and grown under continuous agitation for 48 h at 25 ºC. After that, the contents of the flasks were transferred to 50 mL tubes and centrifuged for 10 min at 890 × *g*. The supernatant of the centrifuged tubes was

then discarded, and the remaining pellet was dissolved in phosphate-buffered saline (PBS, GIBCO). Bacterial concentration was read at 600 nm and adjusted to 1×10^8 colony-forming unit (CFU) mL^{-1}. Fish were then inoculated with the bacteria through peritoneal injection with 100 µL of the above suspension (1×10^7 CFU $fish^{-1}$) and cumulative mortality was followed for eight days. Fish were daily fed the same diets as the growth trial. Dead and moribund fish were collected/euthanized. Any survivors at the end of this challenge were euthanized by an anesthetic overdose (1 mL L^{-1} of 2-phenoxyethanol).

2.7. Chemical analysis

The proximate analysis of the marine hydrolysate and diets was performed in duplicate, according to the AOAC methods (AOAC, 2005). Dry matter (DM) was determined using a drying oven (Binder ED 53; Tuttlingen, Germany; 105 °C for 24 h); ash was determined by combustion in a muffle furnace (Nabertherm L9/11/B170; Bremen, Germany; 550 °C for 6 h); crude protein (CP; N × 4.9 for the hydrolysate (Mæhre et al., 2018) and N × 6.25 for the diets and whole-body composition (Mariotti et al., 2008)) was measured using a nitrogen analyzer (Leco FP-528; St. Joseph, USA); crude fat (CF) was evaluated by petroleum ether (40–60 °C) extraction (Foss Soxtec™ 2055; Höganäs, Sweden), and gross energy was determined using an adiabatic bomb calorimeter (IKA Werke C2000; Staufen, Germany) calibrated with benzoic acid.

For the determination of whole-body composition, the previously collected fish were ground and pooled. Afterwards, samples were freeze-dried and further analyzed for dry matter, ash, crude protein, crude fat, and gross energy following the methodology described above.

2.8. Stress and nutritional status

Cortisol was extracted from 20 µL of plasma in 180 µL of diethyl ether (Sigma-Aldrich, MO, USA) and determined using a commercial ELISA kit (RE52061; IBL International GMBH, Hamburg, Germany).

The plasma concentration of total protein was determined using a commercial kit (Ref. 1001290; Spinreact, Barcelona, Spain). Afterwards, plasma was deproteinized with 0.6 M perchloric acid and neutralized with 1 M potassium bicarbonate prior to being centrifuged at 13500 × *g* for 4.5 min at 4 °C. Then, levels of glucose, cholesterol, triglycerides, and lactate were determined enzymatically using commercial kits (Ref. 1001190, Ref. 1001090, Ref. 1001310, and Ref. 1001330, respectively; Spinreact, Barcelona, Spain). All analyses were conducted in duplicates and absorbances read in a microplate reader.

2.9. Plasma humoral immune parameters

Plasma lysozyme, peroxidase, and alternative complement pathway (ACH50) activities were determined as described in Costa et al. (2020), with triplicate measurements in a microplate spectrophotometer (BioTek Synergy HT, VT, USA). Plasma lysozyme activity (μg mL^{-1}) was determined using a turbidimetric assay adapted to microplate, using a calibration curve with serially diluted lyophilized hen egg-white lysozyme (Sigma-Aldrich, MO, USA) in sodium phosphate buffer (0.05 M, pH 6.2) (Hutchinson and Manning, 1996). For determination of the peroxidase activity (EU mL^{-1}), 5 μL of plasma were diluted with 145 μL of Hanks' Balanced Salt Solution (HBSS), without Ca^{2+} and Mg^{2+}, in a microplate. Next, 50 μL of 20 mM 3,3',5,5'-tetramethylbenzidine hydrochloride (Sigma-Aldrich, MO, USA) and 50 μL of 5 mM hydrogen peroxide were added. After 2 min, the color-change reaction was halted with 50 μL of 2 M sulfuric acid, and the optical density read at 450 nm. One peroxidase enzyme unit (EU) was defined as the amount which causes an absorbance change of one optical density (OD) (Quade and Roth, 1997). The determination of the ACH50 activity (units mL^{-1}) was based on the lysis of rabbit red blood cells (Probiológica, Lisboa, Portugal), using a concentration of 2.8×10^8 cells mL^{-1}. ACH50 units were set as the concentration of plasma that caused a cell lysis of 50% (Sunyer and Tort, 1995).

2.10. Calculations

The following formulas for the determination of growth performance and feed efficiency were used: Specific growth rate (SGR) = 100 × [ln (final body weight [g]) - ln (initial body weight [g])] / trial duration (d); Average body weight (ABW) = (initial body weight [kg] + final body weight [kg]) / 2; Voluntary feed intake (VFI) = (100 × (dry feed

intake [g]) / (average body weight [kg])) / trial duration [d]; Weight gain (WG) = final body weight (g) - initial body weight (g); Condition factor (K) = 100 × (final body weight [g] / final body length [cm]^3); Feed conversion ratio (FCR) = dry feed intake (g) / weight gain (g); Protein efficiency ratio (PER) = weight gain (g) / crude protein intake (g); Daily growth index (DGI) = 100 × [(final body weight [g]$^{1/3}$) - (initial body weight [g]$^{1/3}$)] / trial duration (d); Hepatosomatic index (HSI) = 100 × (liver weight [g] / final body weight [g]); Viscerosomatic index (VSI) = 100 × (viscera weight [g] / final body weight [g]).

To determine the cumulative mortality in the infectious challenge the following formula was used: Mortality = 100 × (number of dead animals / total number of animals).

2.11. Statistical analysis

Results are expressed as mean ± standard error (SE). Data were tested for normality and homogeneity of variances by Shapiro-Wilk and Levene's tests, respectively, and when necessary, appropriately transformed. An independent-sample t-test, used for the zootechnical parameters and whole-body composition analysis, and a two-way ANOVA test, used for the stress challenge considering the diet and stress as fixed factors, were used to analyze the data, with the SPSS Statistics software (version 28.0.1.0; IBM, IL, USA). When using the two-way ANOVA test, whenever significant effects of treatments were detected, means were compared through Tukey's HSD post hoc test. Regarding the infectious challenge, a chi-square test (SPSS Statistics software) was performed to identify differences in cumulative mortality among dietary treatments. Significant differences for all analyses were considered for a $P < 0.05$.

3. Results

3.1. Growth performance

No statistically significant differences were found between the two dietary treatments, CTRL and SHARK, regarding the growth performance parameters (FBW, FBL, WG, K, DGI, VFI, FCR, and SGR) and somatic indices (HSI, and VSI). The only exception was the protein efficiency ratio (PER) (*P*-value = 0.019), that in the CTRL treatment achieved a higher value when compared with the SHARK treatment (Table 3.1).

Table 3.1 – Growth performance parameters of European seabass fed the experimental diets.

	Diets		*P*-value
	CTRL	**SHARK**	
Growth performance			
FBW (g)	52.4 ± 0.65	53.5 ± 0.79	0.280
FBL (cm)	16.3 ± 0.06	16.4 ± 0.08	0.269
WG (g)	39.4 ± 1.41	40.4 ± 1.91	0.699
K	1.2 ± 0.01	1.2 ± 0.003	0.842
DGI	1.6 ± 0.04	1.6 ± 0.05	0.672
VFI	1.4 ± 0.03	1.5 ± 0.03	0.409
FCR	1.04 ± 0.01	1.05 ± 0.003	0.134
SGR	1.6 ± 0.03	1.6 ± 0.04	0.644
PER	1.83 ± 0.01[a]	1.78 ± 0.003[b]	0.019
Somatic indices (%)			
HSI	1.4 ± 0.05	1.4 ± 0.04	0.374
VSI	7.3 ± 0.19	7.7 ± 0.25	0.140

Values are presented as mean ± SE (n = 3, except for FBW and FBL where n = 150, and HSI and VSI where n = 30); Different superscript letters represent significant differences among diets ($P < 0.05$; independent-sample t-test). FBW – Final body weight; FBL – Final body length; WG – Weight gain; K – Condition factor; DGI – Daily growth index; VFI – Voluntary feed intake; FCR – Feed conversion ratio; SGR – Specific growth rate; PER – Protein efficiency ratio; HSI – Hepatosomatic index; VSI – Viscerosomatic index.

3.2. Whole-body composition

Regarding the whole-body composition analysis, no statistically significant differences were found between the two different dietary treatments (CTRL and SHARK), considering the final dry matter, crude protein, crude fat, and gross energy of the fish. Only the ash content presented significant statistical differences (*P*-value = 0.017), achieving in the SHARK treatment a higher amount when compared with the CTRL treatment (Table 3.2).

Table 3.2 – Whole-body composition of European seabass fed the experimental diets.

	Diets		*P*-value
	CTRL	**SHARK**	
Final whole-body composition (% WW)			
Dry matter	36.6 ± 0.34	36.4 ± 0.21	0.546
Ash	3.2 ± 0.09^{b}	3.8 ± 0.11^{a}	0.017
Crude protein	17.1 ± 0.35	17.1 ± 0.11	0.959
Crude fat	14.9 ± 0.62	15.0 ± 0.13	0.960
Gross energy (kJ g^{-1})	10.2 ± 0.16	9.6 ± 0.19	0.074

Values are presented as mean ± SE (n = 3). Different superscript letters indicate significant differences between treatments ($P < 0.05$; independent-sample t-test). Initial whole-body composition (% or kJ g^{-1} wet weight (WW)): Dry matter – 30.24; Ash – 4.44; Crude protein – 16.35; Crude fat – 9.41; Gross energy – 7.39.

3.3. Stress and nutritional status

Concerning the analyzed plasma metabolites, cortisol, and triglycerides levels were both statistically significantly affected by the dietary treatments (*P*-value = 0.01 and *P*-value = 0.02, respectively), with the SHARK treatment achieving lower levels of those metabolites. Additionally, cortisol, glucose, and lactate were significantly increased in stressed fish (*P*-value < 0.001). In contrast, total protein levels were statistically significantly reduced in stressed fish (*P*-value < 0.001).

Lactate was the only plasma metabolite significantly affected by the interaction between diet and stress (*P*-value = 0.03), with higher levels in the SHARK treatment on stressed fish. On the contrary, cholesterol was unaffected by either diet or stress (Table 3.3).

Table 3.3 – Plasma metabolite levels of fish fed the experimental diets, before (non-stressed) or after (stressed) a stress challenge.

	Diets						
	Non-stressed		**Stressed**		***P*-value**		
	CTRL	**SHARK**	**CTRL**	**SHARK**	**Diet**	**Stress**	**Diet x Stress**
Cortisol (ng mL^{-1})	456.06 ± 45.77^{a}	332.22 ± 26.04^{b}	741.59 ± 30.33^{a}	630.81 ± 59.95^{b}	0.01	<0.001	0.89
Glucose (mmol L^{-1})	5.80 ± 0.34	5.32 ± 0.53	7.70 ± 0.50	7.53 ± 0.46	0.48	<0.001	0.74
Lactate (mmol L^{-1})	3.64 ± 0.31^{BC}	3.02 ± 0.18^{C}	4.84 ± 0.45^{AB}	5.83 ± 0.34^{A}	0.60	<0.001	0.03
Triglycerides (mmol L^{-1})	4.84 ± 0.55^{a}	3.60 ± 0.52^{b}	4.87 ± 0.59^{a}	3.42 ± 0.49^{b}	0.02	0.89	0.85
Cholesterol (mmol L^{-1})	3.39 ± 0.32	3.58 ± 0.25	3.41 ± 0.19	2.84 ± 0.26	0.31	0.37	0.09
Total protein (g dL^{-1})	4.15 ± 0.27	4.35 ± 0.15	3.63 ± 0.18	3.09 ± 0.27	0.46	<0.001	0.11

Values are presented as mean ± SE (n = 9). Different superscript lowercase letters denote significant differences among diets, while different superscript uppercase letters indicate significant differences for Diet x Stress (*P* < 0.05; two-way ANOVA).

3.4. Plasma humoral immune parameters

Regarding the innate immune markers, ACH50 was unaffected by either stress or dietary treatments. Lysozyme was not affected by the diet, but it was significantly affected by stress (*P*-value = 0.01), achieving higher levels in the stressed fish. Peroxidase was the only immune parameter statistically significantly affected by the interaction of both diet and stress (*P*-value = 0.01), achieving higher levels in the CTRL treatment on stressed fish (Table 3.4).

Table 3.4 – Humoral immune parameters of fish fed the experimental diets, before (non-stressed) or after (stressed) a stress challenge.

	Diets						
	Non-stressed		**Stressed**		***P*-value**		
	CTRL	**SHARK**	**CTRL**	**SHARK**	**Diet**	**Stress**	**Diet x Stress**
Lysozyme (μg mL^{-1})	22.41 ± 3.10	21.17 ± 2.59	27.75 ± 1.40	30.63 ± 3.09	0.76	0.01	0.45
Peroxidase (EU mL^{-1})	121.74 ± 15.67^{B}	96.86 ± 22.73^{B}	288.13 ± 30.21^{A}	129.73 ± 23.82^{B}	<0.001	<0.001	0.01
ACH50 (units mL^{-1})	132.54 ± 21.22	176.66 ± 21.14	150.75 ± 18.75	170.44 ± 17.77	0.09	0.56	0.54

Values are presented as mean ± SE (n = 9). Different superscript uppercase letters denote significant differences for Diet x Stress ($P < 0.05$; two-way ANOVA).

3.5. Infectious challenge

After performing the infectious challenge, no statistically significant differences were found between the CTRL and SHARK treatments (*P*-value = 0.273). From day 4-6 both treatments seem to have an equal effect on the cumulative mortality of fish. However, even though there were no statistical differences, from day 1-4 and day 6-8 the cumulative mortality of the SHARK treatment was lower than the CTRL treatment (Figure 3.1).

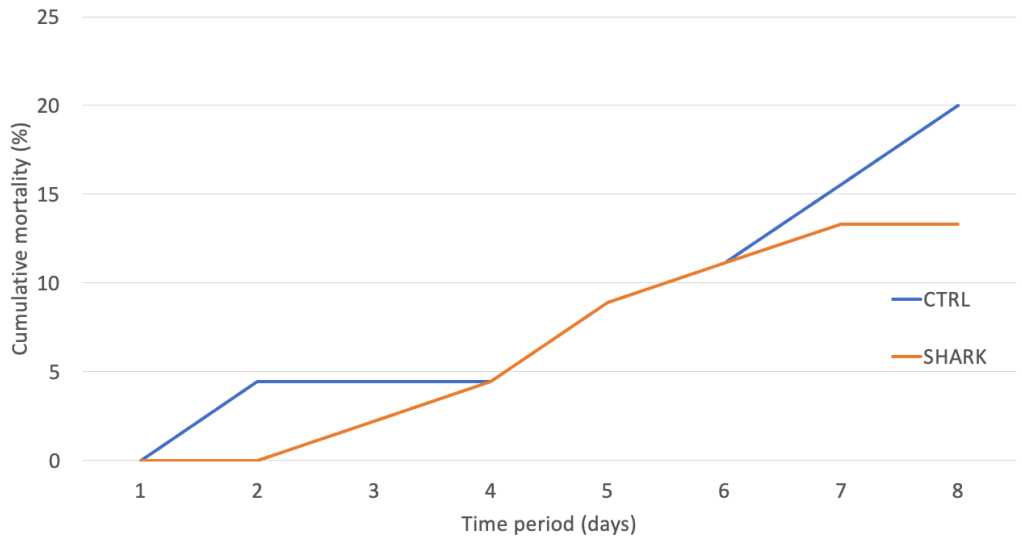

Figure 3.1 – Cumulative mortality (%) of European seabass (n = 45) fed the experimental diets, after a challenge with *Photobacterium damselae* subsp. *piscicida*. No statistical differences were found ($P < 0.05$; chi-square test).

4. Discussion

Recent studies have demonstrated the potential of using hydrolysates as a strategy to minimize the negative impacts of increased protein from plant sources in aquafeeds (Costa et al., 2020; Gunathilaka et al., 2021), thus creating functional diets. In addition, the use of hydrolysates obtained from by-products of the agri-food industry offers both economic and environmental benefits. Indeed, it allows to increase the utilization of animal by-products, leading to a potential income generation, a reduction of environmental impact, and a decrease in disposal costs (Martínez-Alvarez et al., 2015).

The positive effects of hydrolysates on fish growth and robustness are mainly due to the presence of bioactive compounds, but their characteristics and potential differ depending on the raw material used and the processing methodology. Therefore, the present study represents a holistic approach to evaluate the potential benefits of shark skin hydrolysates to cope with both stress conditions and disease outbreaks faced on aquaculture farms. It is important to state that there is no information, to the best of my knowledge, on the use of MPHs obtained through the enzymatic hydrolysis of blue shark skin in diets for European seabass. This reinforces the novelty of the present work, contributing to the increasing literature on this topic.

Regarding the growth performance parameters, overall, there were no significant statistical differences between treatments in the present work. This demonstrates that the experimental SHARK diet was well accepted by the fish and provided sufficient nutrients and energy to meet growth and health requirements of European seabass, despite the 24% reduction in fishmeal (FM). Furthermore, this is also corroborated by the fact that the somatic indices showed no detrimental effect on fish fed the experimental diet. The only exception was the protein efficiency ratio (PER), which in the SHARK treatment achieved a lower value than the CTRL treatment. As evidenced by Hevrøy et al. (2005) in a study on Atlantic salmon (*Salmo salar*) where they found a linear decrease in PER with an increasing inclusion rate of protein hydrolysates, it is possible that the SHARK group is using the proteins and amino acids for energy generation instead of growth, which would negatively affect feed utilization, resulting in several implications such as: i) slower growth rates or even delayed growth due to inadequate protein utilization; ii) higher feed requirements to meet their protein needs, leading to higher feed costs; iii)

imbalances in other essential nutrients such as amino acids, vitamins or minerals, since dietary protein is not used effectively; iv) and finally, a low PER can lead to poor FCR, which means that a higher amount of feed is required to produce the same fish biomass (Hevrøy et al., 2005; Ullah-Khan et al., 2019). This negative repercussion was observed in the study carried out in the same species by Resende et al. (2022), in which it was observed that the inclusion of 3% of swine blood hydrolysate obtained by auto-hydrolysis, decreased PER levels compared to a control group with 12.5% of FM. This resulted in a worse FCR, FBW, and FBL, in spite of presenting similar VFI values. However, in the present study, the group with the lowest PER did not present any negative impact on the rest of the parameters, since, as mentioned above, the other growth performance indicators were not significantly different from the CTRL treatment and the animals grew as expected, quadrupling their weight in around 13 weeks. This suggests that fish fed the SHARK diet compensated for the lower protein utilization through other mechanisms, such as modification of their metabolism, or the presence of other dietary components, such as bioactive peptides, may have modulated the mechanisms involved in muscle growth (Velasco et al., 2023), improving the overall efficiency of nutrient utilization and allowing for normal growth. It is important to note that PER is only one measure of protein utilization, and there are other factors that can influence growth and nutrient utilization in fish. Therefore, understanding these mechanisms and their implications is essential to optimize aquafeeds, ensure adequate nutrition and promote healthy growth of fish.

Marine hydrolysates contain bioactive peptides that have growth-promoting and nutrient utilization effects, among others (Siddik et al., 2021a; Nguyen et al., 2023). Although they differ among studies depending on the source, the type of hydrolysate obtained, the percentage of hydrolysate inclusion, as well as the fish species (Costa et al., 2020), the positive results found in this study, after replacing a low percentage of FM with hydrolysates of marine origin in a diet with high vegetable content, are in agreement with other studies conducted in fish fed diets incorporating MPHs. For example, FM was successfully replaced by MPHs at low levels in diets for Atlantic salmon (Egerton et al., 2020), tiger puffer (*Takifugu rubripes*) (Wei et al., 2021), and red seabream (Bui et al., 2014; Khosravi et al., 2015). Even, significantly better results than those have been reported in the study conducted by Suma et al. (2023) in which they evidenced that fish growth and feed efficiency (FBW, WG, SGR, FCR, K, HSI, and VSI) of juvenile pabda

improved with a 8% FM diet supplemented with 2% commercial hydrolysate at the expense of FM, compared to a control diet with 10% FM. Focusing on the specific case of European seabass and considering exclusively hydrolysates of marine origin, Gisbert et al. (2018) found no differences in the growth performance parameters (FBW, FBL, SGR, and K) when using an experimental diet with a 15% of FM and inclusion of 5% shrimp hydrolysate (at the expense of FM), when compared to the control diet with a higher level of FM (20%). Similarly, Parma et al. (2023) found no significant differences in the growth performance parameters and nutritional and somatic indices using a diet with 10% FM and 5% hydrolyzed Atlantic salmon by-products (at the expense of FM), compared to the control diet with 15% FM. Interestingly, Leduc et al. (2018a,b), demonstrated that a 15% decrease in FM impairs growth performance, but if this decrease in FM percentage is accompanied by the inclusion of 5% MPHs (5% FM + 5% MPHs) it restored growth performance to the same level as the control diet with 20% FM. In this case, the results are significantly positive, even using lower FM levels than those of the present study, which shows that this small difference of 2% in the inclusion of the hydrolysate (5%) with respect to the present study (3%) can be decisive.

Concerning the whole-body composition analysis, in the present study there were differences related to the ash content, that in the SHARK treatment achieved a higher quantity than the CTRL treatment. In several studies, in which a small inclusion level of hydrolysates from different sources was used, no alteration in the final composition of juvenile European seabass was observed, as demonstrated by Resende et al. (2022) using swine blood hydrolysates, or Costa et al. (2020) and Gisbert et al. (2018) using marine hydrolysates. The same seems to happen in other fish species. For instance, Khosravi et al. (2015) found no effect on the whole-body composition of juvenile red seabream and olive flounder (*Paralichthys olivaceus*) fed a 2% krill or tuna hydrolysate. Wei et al. (2021) found no difference in the whole-body composition of juvenile tiger puffer fed a diet with 5% pollock hydrolysates. However, Egerton et al. (2020) found differences in terms of the whole-body composition of juvenile Atlantic salmon, specifically in the ash content, but in this case, showing lower quantity than in the control diet. Marine hydrolysates have a variable mineral content (Folador et al., 2006), so when they are included in fish feeds those differences in minerals can contribute, as is probably happening in the present study, to the increased ash content of fish. Also, minerals in the SHARK diet can be in a form that is more bioavailable and digestible for fish, so they are

more easily absorbed. Hydrolysates may also have metabolic effects that influence mineral availability, utilization, and deposition in fish, enhancing mineral absorption by improving gut health, regulating mineral metabolism, or affecting mineral deposition in various tissues (Sun et al., 2020; Lall and Kaushik, 2021). As discussed above, in the same way, the source and the process of obtaining the hydrolysates influence the bioactivity of the products, they will also be key factors in determining the compositional characteristics of the hydrolysates and therefore their mineral content and profile.

However, while fish growth and whole-body composition are important parameters for assessing the performance of a new formulation, they should not be the only criteria. It is also necessary to study the metabolic response of the fish to ensure that any dietary modifications do not lead to significant metabolic alterations that could potentially impact the fish physiological well-being and adaptability, ensuring not only their growth but also their long-term health. The results obtained in the present study showed that triglycerides were significantly affected by the dietary treatments, obtaining lower levels with the SHARK treatment. This could demonstrate that the hydrolysate obtained from shark skin positively modulates lipid metabolism by regulating lipid sensing mechanisms and promoting better lipid utilization, thus reducing plasma triglycerides levels in fish (Librán-Pérez et al., 2012,2013). Interestingly, in the same way, it was observed a significant decrease in plasma cortisol levels in fish fed the SHARK treatment. This fact is evidence that the bioactive compounds present in the SHARK treatment help to improve the stress response, which in physiological terms can be very positive since fish fed with this diet do not need to mobilize the same amount of energy to cope with challenging situations, being able to use that energy in more important physiological processes, such as growth and reproduction. Following the stress-induced challenge, cortisol, glucose, and lactate were all affected, achieving higher levels in the stressed fish, thus validating the experimental design since those are typical indicators of acute stress. Total protein level was also affected by stress, achieving lower levels in stressed fish, as previously reported (Di Marco et al., 2008; Fernández-Alacid et al., 2019; Parma et al., 2023; Resende et al. 2023; Samaras et al., 2023), probably due to the proteolysis resulting from cortisol action (Di Marco et al., 2008; Pelusio et al., 2022). Lactate was the only plasma metabolite that presented an interaction between diet and stress, with the highest value obtained in the SHARK treatment after induced stress. This may indicate that the SHARK treatment stimulates anaerobic glycolysis (the process that

produces lactate from glucose) in response to a stressor, which can be detrimental to fish health and well-being (Resende et al., 2023). In other species, different effects are seen. For example, Khosravi et al. (2015) found no effect on the nutritional status parameters of juvenile red seabream and olive flounder fed a protein hydrolysate. However, Suma et al. (2023) found a considerable rise in glucose and total protein of juvenile pabda. Since marine hydrolysates contain bioactive peptides that may modulate glucose, lipid, and protein metabolism, as well as hormone regulation (Siddik et al., 2021a; Parma et al., 2023; Suma et al., 2023), it is important to verify that these alterations are positive, improving metabolic status and response to potential stressors.

The humoral non-specific immune parameters of European seabass analyzed in this study – lysozyme, peroxidase, and ACH50, are common indicators of the innate immune status in fish (Campos, 2019). Lysozyme hydrolyzes peptidoglycan residues in bacterial cell walls (Buonocore et al., 2014), while peroxidase is involved in the oxidative response against pathogens (Alvarez-Pellitero, 2008), and the ACH50 pathway comprises a cascade of proteins, activated after contact with a pathogen, resulting in phagocytosis, inflammation, or membrane disruption of the pathogen (Boshra et al., 2006). Several studies demonstrated that hydrolysates contain bioactive peptides that can interact with the immune system of fish, leading to enhanced immune responses (Siddik et al., 2021a; Nguyen et al., 2023). Specifically, the stimulatory effect of dietary hydrolysates on the immune system has been verified by the increased plasma lysozyme activity in different studies in juvenile European seabass (Gisbert et al., 2018; Costa et al., 2020), and red seabream (Khosravi et al., 2015). In the present study, lysozyme was significantly influenced by the stress-induced challenge, being higher in stressed fish, which could demonstrate activation of the immune response to a challenge. In addition, an interaction between diet and stress was observed in peroxidase, which reached a significantly higher value in the CTRL treatment after stress. This may indicate that the CTRL treatment promotes higher immunomodulation, considered a positive effect, by possibly activating immune cells, such as macrophages and neutrophils, which are involved in the production and release of peroxidase (Faurschou and Borregaard, 2003; Machado et al., 2018). However, Resende et al. (2022) found that the inclusion of 3% swine blood hydrolysates did not increase significantly the innate immune parameters in the plasma of juvenile European seabass. This fact reinforces the previously stated idea, that the effect of protein hydrolysates on humoral non-specific immune parameters may vary due to several

factors, such as the hydrolysate source, molecular weights, variations in the peptide profiles, and amino acid compositions (Carvalho et al., 2004; Swanepoel and Goosen, 2018; Wu et al., 2018). Additionally, the quality and processing methods used to produce the hydrolysates can influence their bioactivity and subsequent effects on the immune system (Bøgwald et al., 1996; Gildberg et al., 1996).

In relation to the infectious challenge with *Photobacterium damselae* subsp. *piscicida*, an important bacterial pathogen of European seabass, no significant differences were found between the two treatments with respect to the cumulative mortality, which was low (<25%). However, it seems that if the infectious challenge was prolonged (longer than the eight days studied) the SHARK treatment would lead to significantly lower cumulative mortality than the CTRL treatment. It is important to state that this is to the best of my knowledge the first work studying the *in vivo* effect of a marine hydrolysate on this specific bacterium. This positive response in terms of mortality in juvenile European seabass was observed in different studies such as the one conducted by Resende et al. (2022), which found a significant increase in the resistance to a challenge with *Tenacibaculum maritimum* after feeding a diet with 3% inclusion of swine blood hydrolysates. Also, Gisbert et al. (2018), found that the inclusion of 5% shrimp hydrolysate in a diet led to better survival rates after an outbreak with *Vibrio pelagius*. Similarly, this positive effect was also reported in other fish species. Siddik et al. (2021b) found a significant reduction in mortality in juvenile Asian seabass, fed a diet with an inclusion of around 5% tuna hydrolysate, following a challenge with *Vibrio harvei*. Khosravi et al. (2015) found that juvenile red seabream and olive flounder fed a diet with the inclusion of 2% krill or tuna hydrolysate increased disease resistance against *Edwardsiella tarda*. Suma et al. (2023) found that the cumulative mortality of juvenile pabda against *Aeromonas hydrophila* was significantly decreased when fish were fed a diet with 2% hydrolysate inclusion. Bioactive peptides found in marine hydrolysates have been proven to have antibacterial and immunomodulatory properties (Siddik et al., 2021a; Nguyen et al., 2023). However, those effects can vary depending on several factors, including the composition and characteristics of the different hydrolysates used. Furthermore, the efficacy of such hydrolysates also varies depending on the bacteria causing the infection. These factors may contribute to the variability in the effectiveness of different marine hydrolysates in reducing mortality rates following an infectious challenge.

5. Conclusions

The present study showed that a low inclusion (3%) of marine hydrolysates from blue shark skin in aquafeeds with high levels of fishmeal replacement by vegetable sources resulted in no apparent significant detrimental impact on the growth performance and overall robustness of juvenile European seabass, when compared to an already in use commercial diet. Therefore, it can be concluded that the hydrolysate used in the present study has promising implications as a circular and functional ingredient for aquafeeds. In addition to being a valid alternative ingredient to fishmeal in terms of acceptability and growth, it can enhance the physiological mechanisms related to immune response, stress modulation, and disease resistance under improved conditions or in different scenarios.

Further research should evaluate the bioactivity and tissue-specific effects of blue shark skin hydrolysate on a variety of fish tissues, such as muscle, gut, liver, and head kidney. Also, the study of the effects of hydrolysate inclusion on a vegetable-based diet for European seabass over extended periods, and associated variations in gut microbiota, would be beneficial to better understand the interaction of dietary nutrients and gut microbiota, and their effects on host health, development, and growth. The effect of the hydrolysate on peptide transporters (e.g., intestinal oligopeptide transporter, PEPT1 – a specific marker of dietary protein absorption) should additionally be considered. Future studies could also examine the hematological profile and the expression of other immunological markers, such as immunoglobulins, antiproteases, or even inflammatory markers, to have a better understanding of the ability of hydrolysates to modulate the immune response.

6. References

Aksnes, A., Hope, B., Jönsson, E., Björnsson, B. T., & Albrektsen, S. (2006). Size-fractionated fish hydrolysate as feed ingredient for rainbow trout (*Oncorhynchus mykiss*) fed high plant protein diets. I: Growth, growth regulation and feed utilization. Aquaculture, 261(1), 305-317. https://doi.org/10.1016/j.aquaculture.2006.07.025.

Al Khawli, F., Martí-Quijal, F. J., Ferrer, E., Ruiz, M. J., Berrada, H., Gavahian, M., Barba, F. J., & de la Fuente, B. (2020). Aquaculture and its by-products as a source of nutrients and bioactive compounds. In Advances in Food and Nutrition Research (Vol. 92, pp. 1-33). Academic Press.

Alvarez-Pellitero, P. (2008). Fish immunity and parasite infections: from innate immunity to immunoprophylactic prospects. Veterinary Immunology and Immunopathology, 126(3-4), 171-198. https://doi.org/10.1016/j.vetimm.2008.07.013.

Andrade, T. C., & Boscolo, W. R. (2019). Bioactive peptides and animal hydrolysate proteins in aquaculture. AQUA Culture Asia Pacific Magazine September/October 2019.

Andreoni, F., & Magnani, M. (2014). Photobacteriosis: prevention and diagnosis. Journal of Immunology Research, 2014. https://doi.org/10.1155/2014/793817.

AOAC. (2005). Official methods of analysis. 18th Edition, Association of Officiating Analytical Chemists, Washington DC.

APROMAR. (2022). La acuicultura en España 2022.

Aragão, C., Gonçalves, A. T., Costas, B., Azeredo, R., Xavier, M. J., & Engrola, S. (2022). Alternative proteins for fish diets: implications beyond growth. Animals, 12(9), 1211. https://doi.org/10.3390/ani12091211.

Ashley, P. J. (2007). Fish welfare: current issues in aquaculture. Applied Animal Behaviour Science, 104(3-4), 199-235. https://doi.org/10.1016/j.applanim.2006.09.001.

Bakopoulos, V., Peric, Z., Rodger, H., Adams, A., & Richards, R. (1997). First report of fish pasteurellosis from Malta. Journal of Aquatic Animal Health, 9(1), 26-33. https://doi.org/10.1577/1548-8667(1997)009<0026:FROFPF>2.3.CO;2.

Baptista, T., Romalde, J. L., & Toranzo, A. E. (1996). First occurrence of pasteurellosis in Portugal affecting cultured gilthead seabream. Bulletin of the European Association of Fish Pathologists, 16(3), 92.

Barton, B. A. (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology, 42(3), 517-525. https://doi.org/10.1093/icb/42.3.517.

Batista, I. (2013). Biological activities of fish-protein hydrolysates. In Marine Proteins and Peptides: Biological Activities and Applications (pp. 111-138). Wiley-Blackwell.

Batista, I., Pires, C., & Nunes, M. L. (2006). Hidrolisados proteicos de pescado: preparação, propriedades e utilizações. Publicações avulsas do IPIMAR.

Batista, M. P., Fernández, N., Gaspar, F. B., do Rosário Bronze, M., & Duarte, A. R. C. (2022). Extraction of biocompatible collagen from blue shark skins through the conventional extraction process intensification using natural deep eutectic solvents. Frontiers in Chemistry, 10. https://doi.org/10.3389/fchem.2022.937036.

Benjakul, S., Yarnpakdee, S., Senphan, T., Halldorsdottir, S. M., & Kristinsson, H. G. (2014). Fish protein hydrolysates: production, bioactivities, and applications. In Antioxidants and Functional Components in Aquatic Foods (pp. 237-281). Wiley-Blackwell.

Bøgwald, J., Dalmo, R., Leifson, R. M., Stenberg, E., & Gildberg, A. (1996). The stimulatory effect of a muscle protein hydrolysate from Atlantic cod, *Gadus morhua* L., on Atlantic salmon, *Salmo salar* L., head kidney leucocytes. Fish and Shellfish Immunology, 6(1), 3-16. https://doi.org/10.1006/fsim.1996.0002.

Boshra, H., Li, J., & Sunyer, J. O. (2006). Recent advances on the complement system of teleost fish. Fish and Shellfish Immunology, 20(2), 239-262. https://doi.org/10.1016/j.fsi.2005.04.004.

Braithwaite, V. A., & Ebbesson, L. O. E. (2014). Pain and stress responses in farmed fish. Revue Scientifique et Technique, 33(1), 245-253. https://doi.org/10.20506/rst.33.1.2285.

Bui, H. T. D., Khosravi, S., Fournier, V., Herault, M., & Lee, K. J. (2014). Growth performance, feed utilization, innate immunity, digestibility and disease resistance of juvenile red seabream (*Pagrus major*) fed diets supplemented with protein hydrolysates. Aquaculture, 418, 11-16. https://doi.org/10.1016/j.aquaculture.2013.09.046.

Buonocore, F., Randelli, E., Trisolino, P., Facchiano, A., de Pascale, D., & Scapigliati, G. (2014). Molecular characterization, gene structure and antibacterial activity of a g-type lysozyme from the European sea bass (*Dicentrarchus labrax* L.). Molecular Immunology, 62(1), 10-18. https://doi.org/10.1016/j.molimm.2014.05.009.

Caldeira, M., Barreto, C., Pestana, P., Cardoso, M. A. T., Franca, Z., Plataforma, I., & Módulo, P. I. K. (2018). Fish residue valorisation by the production of value-added compounds towards a sustainable zero waste industry: A critical review. Journal of Scientific and Engineering Research, 5, 418-447.

Campos, I. G. (2019). Processed animal by-products as sustainable ingredients in diets for European seabass (*Dicentrarchus labrax*) (Doctoral dissertation, Universidade do Porto, Portugal).

Cardoso, C., & Nunes, M. L. (2013). Improved utilization of fish waste, discards, and by-products and low-value fish towards food and health products. In Utilization of Fish Waste (pp. 26-58). CRC Press.

Carvalho, A. P., Sá, R., Oliva-Teles, A., & Bergot, P. (2004). Solubility and peptide profile affect the utilization of dietary protein by common carp (*Cyprinus carpio*) during early larval stages. Aquaculture, 234(1-4), 319-333. https://doi.org/10.1016/j.aquaculture.2004.01.007.

Chaklader, M. R., Fotedar, R., Howieson, J., Siddik, M. A., & Foysal, M. J. (2020). The ameliorative effects of various fish protein hydrolysates in poultry by-product meal based diets on muscle quality, serum biochemistry and immunity in juvenile barramundi, *Lates calcarifer*. Fish and Shellfish Immunology, 104, 567-578. https://doi.org/10.1016/j.fsi.2020.06.014.

Chi, C. F., Wang, B., Hu, F. Y., Wang, Y. M., Zhang, B., Deng, S. G., & Wu, C. W. (2015). Purification and identification of three novel antioxidant peptides from protein hydrolysate of bluefin leatherjacket (*Navodon septentrionalis*) skin. Food Research International, 73, 124-129. https://doi.org/10.1016/j.foodres.2014.08.038.

Chotikachinda, R., Tantikitti, C., Benjakul, S., Rustad, T., & Kumarnsit, E. (2013). Production of protein hydrolysates from skipjack tuna (*Katsuwonus pelamis*) viscera as feeding attractants for Asian seabass (*Lates calcarifer*). Aquaculture Nutrition, 19(5), 773-784. https://doi.org/10.1111/anu.12024.

Conde-Sieira, M., Gesto, M., Batista, S., Linares, F., Villanueva, J. L., Míguez, J. M., Soengas, J. L., & Valente, L. M. (2018). Influence of vegetable diets on physiological and immune responses to thermal stress in Senegalese sole (*Solea senegalensis*). PLoS One, 13(3), e0194353. https://doi.org/10.1371/journal.pone.0194353.

Coppola, D., Lauritano, C., Palma Esposito, F., Riccio, G., Rizzo, C., & de Pascale, D. (2021). Fish waste: From problem to valuable resource. Marine Drugs, 19(2), 116. https://doi.org/10.3390/md19020116.

Costa, M., Costas, B., Machado, M., Teixeira, C., Fernández-Boo, S., Sá, T., Batista, S., Marques, A., Miranda, F., & Valente, L. M. P. (2020). Anchovy and giant squid hydrolysates can enhance growth and the immune response of European seabass (*Dicentrarchus labrax*) fed plant-protein-based diets. Aquaculture, 523, 735182. https://doi.org/10.1016/j.aquaculture.2020.735182.

Daniel, N. (2018). A review on replacing fish meal in aqua feeds using plant protein sources. International Journal of Fisheries and Aquatic Studies, 6(2), 164-179.

Davis, K. B. (2006). Management of physiological stress in finfish aquaculture. North American Journal of Aquaculture, 68(2), 116-121. https://doi.org/10.1577/A05-007.1.

Di Marco, P., Priori, A., Finoia, M. G., Massari, A., Mandich, A., & Marino, G. (2008). Physiological responses of European sea bass *Dicentrarchus labrax* to different stocking densities and acute stress challenge. Aquaculture, 275(1-4), 319-328. https://doi.org/10.1016/j.aquaculture.2007.12.012.

Duarte, C. M., Holmer, M., Olsen, Y., Soto, D., Marbà, N., Guiu, J., Black, K., & Karakassis, I. (2009). Will the oceans help feed humanity?. BioScience, 59(11), 967- 976. https://doi.org/10.1525/bio.2009.59.11.8.

Egerton, S., Culloty, S., Whooley, J., Stanton, C., & Ross, R. P. (2018). Characterization of protein hydrolysates from blue whiting (*Micromesistius poutassou*) and their application in beverage fortification. Food Chemistry, 245, 698-706. https://doi.org/10.1016/j.foodchem.2017.10.107.

Egerton, S., Wan, A., Murphy, K., Collins, F., Ahern, G., Sugrue, I., Busca, K., Egan, F., Muller, N., Whooley, J., McGinnity, P., Culloty, S., Ross, R. P., & Stanton, C. (2020). Replacing fishmeal with plant protein in Atlantic salmon (*Salmo salar*) diets by supplementation with fish protein hydrolysate. Scientific Reports, 10(1), 1-16. https://doi.org/10.1038/s41598-020-60325-7.

Ellis, T., Yildiz, H. Y., López-Olmeda, J., Spedicato, M. T., Tort, L., Øverli, Ø., & Martins, C. I. (2012). Cortisol and finfish welfare. Fish Physiology and Biochemistry, 38, 163-188. https://doi.org/10.1007/s10695-011-9568-y.

Eroldoğan, O. T., Glencross, B., Novoveska, L., Gaudêncio, S. P., Rinkevich, B., Varese, G. C., de Fátima Carvalho, M., Tasdemir, D., Safarik, I., Nielsen, S. L., Rebours, C., Lada, L. B., Robbens, J., Strode, E., Haznedaroğlu, B. Z., Kotta, J., Evliyaoğlu, E., Oliveira, J., Girão, M., Vasquez, M. I., Čabarkapa, I., Rakita, S., Klun, K., & Rotter, A. (2022). From the sea to aquafeed: A perspective overview. Reviews in Aquaculture. https://doi.org/10.1111/raq.12740.

Espe, M., Lemme, A., Petri, A., & El-Mowafi, A. (2007). Assessment of lysine requirement for maximal protein accretion in Atlantic salmon using plant protein diets. Aquaculture, 263(1-4), 168-178. https://doi.org/10.1016/j.aquaculture.2006.10.018.

Espe, M., Sveier, H., Høgøy, I., & Lied, E. (1999). Nutrient absorption and growth of Atlantic salmon (*Salmo salar* L.) fed fish protein concentrate. Aquaculture, 174(1-2), 119-137. https://doi.org/10.1016/S0044-8486(98)00502-X.

EUMOFA. (2022). The EU fish market—2022 edition.

European Commission. (2013). Regulation (EU) No 1380/2013 of the European Parliament and of the Council of 11 December 2013 on the Common Fisheries Policy, amending Council Regulations (EC) No 1954/2003 and (EC) No 1224/2009 and repealing Council Regulations (EC) No 2371/2002 and (EC) No 639/2004 and Council Decision 2004/585/EC. Official Journal of the European Union, 354, 22-61.

European Commission. (2020). Communication from the commission to the European parliament, the European council, the council, the European economic and social committee and the committee of the regions. A new Circular Economy Action Plan. For a cleaner and more competitive Europe 1–19. European Commission Brussels. COM(2020) 98 final.

FAO. (1988). Definition of aquaculture, Seventh Session of the IPFC Working Party of Expects on Aquaculture, IPFC/WPA/WPZ.

FAO. (2022). The state of world fisheries and aquaculture 2022. Towards blue transformation. Rome, FAO. https://doi.org/10.4060/cc0461en.

FAO. (2023a). *Prionace glauca* Linnaeus,1758. Fisheries and Aquaculture Division. Rome. https://www.fao.org/fishery/en/aqspecies/2018/en.

FAO. (2023b). *Dicentrarchus labrax*. Cultured aquatic species information programme. Fisheries and Aquaculture Division. Rome. https://www.fao.org/fishery/en/culturedspecies/dicentrarchus_labrax?lang=en.

FAO. (2023c). *Dicentrarchus labrax* Linnaeus,1758. Fisheries and Aquaculture Division. Rome. https://www.fao.org/fishery/en/aqspecies/2291/en.

Faurschou, M., & Borregaard, N. (2003). Neutrophil granules and secretory vesicles in inflammation. Microbes and Infection, 5(14), 1317-1327. https://doi.org/10.1016/j.micinf.2003.09.008.

Fernández-Alacid, L., Sanahuja, I., Ordóñez-Grande, B., Sánchez-Nuño, S., Herrera, M., & Ibarz, A. (2019). Skin mucus metabolites and cortisol in meagre fed acute stress-attenuating diets: Correlations between plasma and mucus. Aquaculture, 499, 185-194. https://doi.org/10.1016/j.aquaculture.2018.09.039.

Folador, J. F., Karr-Lilienthal, L. K., Parsons, C. M., Bauer, L. L., Utterback, P. L., Schasteen, C. S., Bechtel, P. J., & Fahey Jr, G. C. (2006). Fish meals, fish components, and fish protein hydrolysates as potential ingredients in pet foods. Journal of Animal Science, 84(10), 2752-2765. https://doi.org/10.2527/jas.2005-560.

Fournier, V., Herault, M., & Hervy, M. (2011). Marine based protein hydrolysate improve the performance of feeds containing a low level of fish meal. International Aquafeed Magazine November-December 2011.

Francis, G., Makkar, H. P., & Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199(3-4), 197-227. https://doi.org/10.1016/S0044-8486(01)00526-9.

Gildberg, A., Bøgwald, J., Johansen, A., & Stenberg, E. (1996). Isolation of acid peptide fractions from a fish protein hydrolysate with strong stimulatory effect on Atlantic salmon (*Salmo salar*) head kidney leucocytes. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 114(1), 97-101. https://doi.org/10.1016/0305-0491(96)00011-9.

Gisbert, E., Fournier, V., Solovyev, M., Skalli, A., & Andree, K. B. (2018). Diets containing shrimp protein hydrolysates provided protection to European sea bass (*Dicentrarchus labrax*) affected by a *Vibrio pelagius* natural infection outbreak. Aquaculture, 495, 136-143. https://doi.org/10.1016/j.aquaculture.2018.04.051.

Gu, R. Z., Li, C. Y., Liu, W. Y., Yi, W. X., & Cai, M. Y. (2011). Angiotensin I-converting enzyme inhibitory activity of low-molecular-weight peptides from Atlantic salmon (*Salmo salar* L.) skin. Food Research International, 44(5), 1536-1540. https://doi.org/10.1016/j.foodres.2011.04.006.

Gudding, R., & Van Muiswinkel, W. B. (2013). A history of fish vaccination: science-based disease prevention in aquaculture. Fish and Shellfish Immunology, 35(6), 1683-1688. https://doi.org/10.1016/j.fsi.2013.09.031.

Guérard, F. (2009). Valorisation des biomasses: l'or des coproduits. Biofutur, 301, 39-41.

Guérard, F., Dufosse, L., De La Broise, D., & Binet, A. (2001). Enzymatic hydrolysis of proteins from yellowfin tuna (*Thunnus albacares*) wastes using Alcalase. Journal of Molecular Catalysis B: Enzymatic, 11(4-6), 1051-1059. https://doi.org/10.1016/S1381-1177(00)00031-X.

Gunathilaka, B. E., Khosravi, S., Herault, M., Fournier, V., Lee, C., Jeong, J. B., & Lee, K. J. (2020). Evaluation of shrimp or tilapia protein hydrolysate at graded dosages in low fish meal diet for olive flounder (*Paralichthys olivaceus*). Aquaculture Nutrition, 26(5), 1592-1603. https://doi.org/10.1111/anu.13105.

Gunathilaka, B. E., Khosravi, S., Shin, J., Shin, J., Herault, M., Fournier, V., & Lee, K. J. (2021). Evaluation of shrimp protein hydrolysate and krill meal supplementation in low fish meal diet for red seabream (*Pagrus major*). Fisheries and Aquatic Sciences, 24(3), 109-120. https://doi.org/10.47853/FAS.2021.e11.

Ha, N., Jesus, G. F. A., Gonçalves, A. F. N., de Oliveira, N. S., Sugai, J. K., Pessatti, M. L., Mouriño, J. L. P., & Fabregat, T. E. H. P. (2019). Sardine (*Sardinella* spp.) protein hydrolysate as growth promoter in South American catfish (*Rhamdia quelen*) feeding: Productive performance, digestive enzymes activity, morphometry and intestinal microbiology. Aquaculture, 500, 99-106. https://doi.org/10.1016/j.aquaculture.2018.10.004.

Haffray, P., Tsigenopoulos, C. S., Bonhomme, F., Chatain, B., Magoulas, A., Rye, M., Triantafyllidis, A., & Triantaphyllidis, C. (2007). European sea bass-*Dicentrarchus labrax*. In Genetics of Domestication, Breeding and Enhancement of Performance of Fish and Shellfish, Viterbo, Italy, 12-17th June, 2006.

Håstein, T., Gudding, R., & Evensen, O. (2005). Bacterial vaccines for fish-an update of the current situation worldwide. Developments in Biologicals, 121, 55-74.

He, S., Franco, C., & Zhang, W. (2013). Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). Food Research International, 50(1), 289-297. https://doi.org/10.1016/j.foodres.2012.10.031.

Heffernan, S., Giblin, L., & O'Brien, N. (2021). Assessment of the biological activity of fish muscle protein hydrolysates using *in vitro* model systems. Food Chemistry, 359, 129852. https://doi.org/10.1016/j.foodchem.2021.129852.

Herault, M., Fournier, V., Magali, H., & Ngoc, N. A. (2012). Fish protein hydrolysates enhance resistance of fish to different types of stress. AQUA Culture Asia Pacific Magazine March/April 2012.

Herault, M., Gunathilaka, B. E., & Lee, K. J. (2020). Functional hydrolysates increase shrimp feed consistency, sustainability and performance. AQUA Culture Asia Pacific Magazine July/August 2020.

Herault, M., Seguin, P., & Kabir, M. (2022). A functional hydrolysate adds value to feeds for omnivorous fish species. AQUA Culture Asia Pacific Magazine September/October 2022.

Hevrøy, E. M., Espe, M., Waagbø, R., Sandnes, K., Ruud, M., & Hemre, G. I. (2005). Nutrient utilization in Atlantic salmon (*Salmo salar* L.) fed increased levels of fish protein hydrolysate during a period of fast growth. Aquaculture Nutrition, 11(4), 301-313. https://doi.org/10.1111/j.1365-2095.2005.00357.x.

Hou, Y., Wu, Z., Dai, Z., Wang, G., & Wu, G. (2017). Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. Journal of Animal Science and Biotechnology, 8, 1-13. https://doi.org/10.1186/s40104-017-0153-9.

Hua, K., Cobcroft, J. M., Cole, A., Condon, K., Jerry, D. R., Mangott, A., Praeger, C., Vucko, M. J., Zeng, C., Zenger, K., & Strugnell, J. M. (2019). The future of aquatic protein: implications for protein sources in aquaculture diets. One Earth, 1(3), 316-329. https://doi.org/10.1016/j.oneear.2019.10.018.

Hutchinson, T. H., & Manning, M. J. (1996). Seasonal trends in serum lysozyme activity and total protein concentration in dab (*Limanda limanda* L.) sampled from Lyme Bay, UK. Fish and Shellfish Immunology, 6(7), 473-482. https://doi.org/10.1006/fsim.1996.0045.

Idowu, A. T., Igiehon, O. O., Idowu, S., Olatunde, O. O., & Benjakul, S. (2021). Bioactivity potentials and general applications of fish protein hydrolysates. International Journal of Peptide Research and Therapeutics, 27, 109-118. https://doi.org/10.1007/s10989-020-10071-1.

Ishak, N. H., & Sarbon, N. M. (2018). A review of protein hydrolysates and bioactive peptides deriving from wastes generated by fish processing. Food and Bioprocess Technology, 11, 2-16. https://doi.org/10.1007/s11947-017-1940-1.

Jackson, A., & Newton, R. W. (2016). Project to model the use of fisheries by-products in the production of marine ingredients with special reference to omega-3 fatty acids EPA and DHA. A report by IFFO and the University of Stirling, 12.

Khantaphant, S., Benjakul, S., & Ghomi, M. R. (2011). The effects of pretreatments on antioxidative activities of protein hydrolysate from the muscle of brownstripe red snapper (*Lutjanus vitta*). LWT-Food Science and Technology, 44(4), 1139-1148. https://doi.org/10.1016/j.lwt.2010.10.009.

Khosravi, S., Bui, H. T. D., Rahimnejad, S., Herault, M., Fournier, V., Jeong, J. B., & Lee, K. J. (2015). Effect of dietary hydrolysate supplementation on growth performance, non-specific immune response and disease resistance of olive flounder (*Paralichthys olivaceus*) challenged with *Edwardsiella tarda*. Aquaculture Nutrition, 21(3), 321-331. https://doi.org/10.1111/anu.12157.

Kır, M., Sunar, M. C., & Gök, M. G. (2019). Acute ammonia toxicity and the interactive effects of ammonia and salinity on the standard metabolism of European sea bass (*Dicentrarchus labrax*). Aquaculture, 511, 734273. https://doi.org/10.1016/j.aquaculture.2019.734273.

Kotzamanis, Y. P., Gisbert, E., Gatesoupe, F. J., Infante, J. Z., & Cahu, C. (2007). Effects of different dietary levels of fish protein hydrolysates on growth, digestive enzymes, gut microbiota, and resistance to *Vibrio anguillarum* in European sea bass (*Dicentrarchus labrax*) larvae. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 147(1), 205-214. https://doi.org/10.1016/j.cbpa.2006.12.037.

Kristinsson, H. G. (2007). Aquatic food protein hydrolysates. In Maximising the Value of Marine By-products (pp. 229-248). Woodhead Publishing.

Kristinsson, H. G., & Rasco, B. A. (2000). Fish protein hydrolysates: production, biochemical, and functional properties. Critical Reviews in Food Science and Nutrition, 40(1), 43-81. https://doi.org/10.1080/10408690091189266.

Krogdahl, Å., Gajardo, K., Kortner, T. M., Penn, M., Gu, M., Berge, G. M., & Bakke, A. M. (2015). Soya saponins induce enteritis in Atlantic salmon (*Salmo salar* L.). Journal of Agricultural and Food Chemistry, 63(15), 3887-3902. https://doi.org/10.1021/jf506242t.

Lall, S. P., & Kaushik, S. J. (2021). Nutrition and metabolism of minerals in fish. Animals, 11(09), 2711. https://doi.org/10.3390/ani11092711.

Leduc, A., Hervy, M., Rangama, J., Delépée, R., Fournier, V., & Henry, J. (2018a). Shrimp by-product hydrolysate induces intestinal myotropic activity in European seabass (*Dicentrarchus labrax*). Aquaculture, 497, 380-388. https://doi.org/10.1016/j.aquaculture.2018.08.009.

Leduc, A., Zatylny-Gaudin, C., Robert, M., Corre, E., Corguille, G. L., Castel, H., Lefevre-Scelles, A., Fournier, V., Gisbert, E., Andree, K. B., & Henry, J. (2018b). Dietary aquaculture by-product hydrolysates: impact on the transcriptomic response of the intestinal mucosa of European seabass (*Dicentrarchus labrax*) fed low fish meal diets. BMC genomics, 19(1), 1-20. https://doi.org/10.1186/s12864-018-4780-0.

Liang, M., Wang, J., Chang, Q., & Mai, K. (2006). Effects of different levels of fish protein hydrolysate in the diet on the nonspecific immunity of Japanese sea bass, *Lateolabrax japonicus* (Cuvieret Valenciennes, 1828). Aquaculture Research, 37(1), 102-106. https://doi.org/10.1111/j.1365-2109.2005.01392.x.

Liaset, B., Lied, E., & Espe, M. (2000). Enzymatic hydrolysis of by-products from the fish-filleting industry; chemical characterisation and nutritional evaluation. Journal of the Science of Food and Agriculture, 80(5), 581-589. https://doi.org/10.1002/(SICI)1097-0010(200004)80:5<581::AID-JSFA578>3.0.CO;2-I.

Librán-Pérez, M., Figueiredo-Silva, A. C., Panserat, S., Geurden, I., Míguez, J. M., Polakof, S., & Soengas, J. L. (2013). Response of hepatic lipid and glucose metabolism to a mixture or single fatty acids: possible presence of fatty acid-sensing mechanisms. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 164(1), 241-248. https://doi.org/10.1016/j.cbpa.2012.09.012.

Librán-Pérez, M., Polakof, S., López-Patiño, M. A., Míguez, J. M., & Soengas, J. L. (2012). Evidence of a metabolic fatty acid-sensing system in the hypothalamus and Brockmann bodies of rainbow trout: implications in food intake regulation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 302(11), R1340-R1350. https://doi.org/10.1152/ajpregu.00070.2012.

Lin, S., & Luo, L. (2011). Effects of different levels of soybean meal inclusion in replacement for fish meal on growth, digestive enzymes and transaminase activities in practical diets for juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. Animal Feed Science and Technology, 168(1-2), 80-87. https://doi.org/10.1016/j.anifeedsci.2011.03.012.

Machado, M., Azeredo, R., Fontinha, F., Fernández-Boo, S., Conceição, L. E., Dias, J., & Costas, B. (2018). Dietary methionine improves the European seabass (*Dicentrarchus labrax*) immune status, inflammatory response, and disease resistance. Frontiers in Immunology, 9, 2672. https://doi.org/10.3389/fimmu.2018.02672.

Machado, M., Castro, C., Oliva-Teles, A., & Costas, B. (2019). Interactive effects of dietary vegetable oil and carbohydrate incorporation on the innate immune response of European seabass (*Dicentrarchus labrax*) juveniles subjected to acute stress. Aquaculture, 498, 171-180. https://doi.org/10.1016/j.aquaculture.2018.08.050.

Mæhre, H. K., Dalheim, L., Edvinsen, G. K., Elvevoll, E. O., & Jensen, I. J. (2018). Protein determination—method matters. Foods, 7(1), 5. https://doi.org/10.3390/foods7010005.

Malcorps, W., Newton, R., & Little, D. (2020). Valorisation of fish by-products. Deliverable 2.7. GAIN - Green Aquaculture INtensification in Europe. EU Horizon 2020 project grant no. 77330. 41 pp.

Mariotti, F., Tomé, D., & Mirand, P. P. (2008). Converting nitrogen into protein—beyond 6.25 and Jones' factors. Critical Reviews in Food Science and Nutrition, 48(2), 177-184. https://doi.org/10.1080/10408390701279749.

Marti-Quijal, F. J., Remize, F., Meca, G., Ferrer, E., Ruiz, M. J., & Barba, F. J. (2020). Fermentation in fish and by-products processing: An overview of current research and future prospects. Current Opinion in Food Science, 31, 9-16. https://doi.org/10.1016/j.cofs.2019.08.001.

Martínez-Alvarez, O., Chamorro, S., & Brenes, A. (2015). Protein hydrolysates from animal processing by-products as a source of bioactive molecules with interest in animal feeding: A review. Food Research International, 73, 204-212. https://doi.org/10.1016/j.foodres.2015.04.005.

Martínez-Porchas, M., Martínez-Córdova, L. R., & Ramos-Enriquez, R. (2009). Cortisol and glucose: reliable indicators of fish stress?. Pan-American Journal of Aquatic Sciences, 158-178.

Mastandrea, L. R. M. (2021). Obtención de hidrolizados biológicos como medio para aprovechar y mejorar la disposición final de los residuos de piel de tiburón azul (*Prionace glauca*) (Doctoral dissertation, Universidad de la República, Uruguay).

Mateus, A. P., Power, D. M., & Canário, A. V. (2017). Stress and disease in fish. In Fish Diseases (pp. 187-220). Academic Press.

Miller, D. B., & O'Callaghan, J. P. (2002). Neuroendocrine aspects of the response to stress. Metabolism-Clinical and Experimental, 51(6), 5-10. https://doi.org/10.1053/meta.2002.33184.

More, S. J. (2020). European perspectives on efforts to reduce antimicrobial usage in food animal production. Irish Veterinary Journal, 73(1), 2. https://doi.org/10.1186/s13620-019-0154-4.

Muniesa, A., Basurco, B., Aguilera, C., Furones, D., Reverté, C., Sanjuan-Vilaplana, A., Jansen, M. D., Brun, E., & Tavornpanich, S. (2020). Mapping the knowledge of the main diseases affecting sea bass and sea bream in Mediterranean. Transboundary and Emerging Diseases, 67(3), 1089-1100. https://doi.org/10.1111/tbed.13482.

Najafian, L., & Babji, A. S. (2012). A review of fish-derived antioxidant and antimicrobial peptides: Their production, assessment, and applications. Peptides, 33(1), 178-185. https://doi.org/10.1016/j.peptides.2011.11.013.

Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Lubchenco, J., Shumway, S. E., & Troell, M. (2021). A 20-year retrospective review of global aquaculture. Nature, 591(7851), 551-563. https://doi.org/10.1038/s41586-021-03308-6.

Ng, W. K., Leow, T. C., & Yossa, R. (2019). Effect of substituting fishmeal with corn protein concentrate on growth performance, nutrient utilization and skin coloration in red hybrid tilapia, *Oreochromis* sp. Aquaculture Nutrition, 25(5), 1006-1016. https://doi.org/10.1111/anu.12918.

Nghia, N. D. (2020). Seafood by-products: a new way from waste to high added value in pharmaceuticals and cosmetics. In Encyclopedia of Marine Biotechnology (pp. 2961-2986). Wiley-Blackwell.

Nguyen, M. C., Fotedar, R., & Giridharan, B. (2023). The effects of fish protein hydrolysate as supplementation on growth performance, feed utilization and immunological response in fish: A review. MATEC Web of Conferences (Vol. 377, p. 01020). https://doi.org/10.1051/matecconf/202337701020.

Nirmal, N. P., Santivarangkna, C., Rajput, M. S., Benjakul, S., & Maqsood, S. (2022). Valorization of fish byproducts: Sources to end-product applications of bioactive protein hydrolysate. Comprehensive Reviews in Food Science and Food Safety, 21(2), 1803-1842. https://doi.org/10.1111/1541-4337.12917.

Novriadi, R., Hermawan, T., Kadari, M., Dikrurrahman, Ibtisam, Herault, M., Fournier, V., & Seguin, P. (2015). Effect of diets containing fish protein hydrolisates on growth and immune performance of Asian seabass (*Lates calcarifer* Bloch) when reared in farm conditions. Omni-Akuatika, Vol. XIV 1 – 12.

NRC. (2011). Nutrient requirements of fish and shrimp. National Academies Press.

Nunan, C. (2022). Ending routine farm antibiotic use in Europe. Achieving responsible farm antibiotic use through improving animal health and welfare in pig and poultry production.

Okes, N., & Sant, G. (2019). An overview of major shark traders, catchers and species. TRAFFIC, Cambridge, UK.

Ospina-Salazar, G. H., Ríos-Durán, M. G., Toledo-Cuevas, E. M., & Martínez-Palacios, C. A. (2016). The effects of fish hydrolysate and soy protein isolate on the growth performance, body composition and digestibility of juvenile pike silverside, *Chirostoma estor.* Animal Feed Science and Technology, 220, 168-179. https://doi.org/10.1016/j.anifeedsci.2016.08.011.

Ovissipour, M., Abedian Kenari, A., Nazari, R., Motamedzadegan, A., & Rasco, B. (2014). Tuna viscera protein hydrolysate: nutritive and disease resistance properties for Persian sturgeon (*Acipenser persicus* L.) larvae. Aquaculture Research, 45(4), 591-601. https://doi.org/10.1111/j.1365-2109.2012.03257.x.

Parma, L., Busti, S., Ciulli, S., Volpe, E., Errani, F., Oterhals, Å., Romarheim, O. H., Aspevik, T., Dondi, F., Gatta, P. P., & Bonaldo, A. (2023). Growth, plasma biochemistry and immune-related gene expression of European sea bass (*Dicentrarchus labrax*) fed bioactive peptides from farmed salmon by-products. Aquaculture, 563, 738982. https://doi.org/10.1016/j.aquaculture.2022.738982.

Pavlidis, M. A., & Mylonas, C. C. (2011). Sparidae: Biology and aquaculture of gilthead sea bream and other species. John Wiley & Sons.

Pelusio, N. F., Bonaldo, A., Gisbert, E., Andree, K. B., Esteban, M. A., Dondi, F., Sabetti, M. C., Gatta, P. P., & Parma, L. (2022). Different fish meal and fish oil dietary levels in European Sea bass: welfare implications after acute confinement stress. Frontiers in Marine Science, 8, 779053. https://doi.org/10.3389/fmars.2021.779053.

Petrova, I., Tolstorebrov, I., & Eikevik, T. M. (2018). Production of fish protein hydrolysates step by step: technological aspects, equipment used, major energy costs and methods of their minimizing. International Aquatic Research, 10, 223-241. https://doi.org/10.1007/s40071-018-0207-4.

Pham, H. D., Siddik, M. A., Le, H. M., Ngo, M. V., Nguyen, M. V., & Francis, D. (2022). Effects of dietary tuna viscera hydrolysate supplementation on growth, intestinal mucosal response, and resistance to *Streptococcus iniae* infection in pompano (*Trachinotus blochii*). Aquaculture Nutrition, 2022. https://doi.org/10.1155/2022/3645868.

Pottinger, T. G. (2008). The stress response in fish-mechanisms, effects and measurement. In Fish Welfare (pp. 32-48). Blackwell Publishing.

Quade, M. J., & Roth, J. A. (1997). A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Veterinary Immunology and Immunopathology, 58(3-4), 239-248. https://doi.org/10.1016/S0165-2427(97)00048-2.

Ramakrishnan, V. V., Hossain, A., Dave, D., & Shahidi, F. (2023). Salmon processing discards: A potential source of bioactive peptides–a review. Preprints.org, 2023040311. https://doi.org/10.20944/preprints202304.0311.v1.

Refstie, S., Olli, J. J., & Standal, H. (2004). Feed intake, growth, and protein utilisation by post-smolt Atlantic salmon (*Salmo salar*) in response to graded levels of fish protein hydrolysate in the diet. Aquaculture, 239(1-4), 331-349. https://doi.org/10.1016/j.aquaculture.2004.06.015.

Resende, D., Costas, B., Sá, T., Golfetto, U., Machado, M., Pereira, M., Pereira, C., Marques, B. Rocha, C. M. R., Pintado M., & Valente, L. M. P. (2022). Innovative swine blood hydrolysates as promising ingredients for European seabass diets: Impact on growth performance and resistance to *Tenacibaculum maritimum* infection. Aquaculture, 561, 738657. https://doi.org/10.1016/j.aquaculture.2022.738657.

Resende, D., Pereira, R., Domínguez, D., Pereira, M., Pereira, C., Pintado, M., Valente, L. M. P., & Velasco, C. (2023). Stress response of European seabass (*Dicentrarchus labrax*) fed plant-based diets supplemented with swine blood hydrolysates. Aquaculture Reports, 30, 101600. https://doi.org/10.1016/j.aqrep.2023.101600.

Reverter, M., Sarter, S., Caruso, D., Avarre, J. C., Combe, M., Pepey, E., Pouyaud, L., Vega-Heredía, S., de Verdal, H., & Gozlan, R. E. (2020). Aquaculture at the crossroads of global warming and antimicrobial resistance. Nature Communications, 11(1), 1870. https://doi.org/10.1038/s41467-020-15735-6.

Robert, M. (2014). Développement d'hydrolysats pour l'alimentation des animaux d'aquaculture: caractérisation moléculaire et fonctionnelle (Doctoral dissertation, Université de Caen, France).

Sadoul, B., Alfonso, S., Cousin, X., Prunet, P., Bégout, M. L., & Leguen, I. (2021). Global assessment of the response to chronic stress in European sea bass. Aquaculture, 544, 737072. https://doi.org/10.1016/j.aquaculture.2021.737072.

Samaras, A., Papandroulakis, N., Lika, K., & Pavlidis, M. (2018). Water temperature modifies the acute stress response of European sea bass, *Dicentrarchus labrax* L.(1758). Journal of Thermal Biology, 78, 84-91. https://doi.org/10.1016/j.jtherbio.2018.09.006.

Samaras, A., Tsoukali, P., Katsika, L., Pavlidis, M., & Papadakis, I. E. (2023). Chronic impact of exposure to low dissolved oxygen on the physiology of *Dicentrarchus labrax* and *Sparus aurata* and its effects on the acute stress response. Aquaculture, 562, 738830. https://doi.org/10.1016/j.aquaculture.2022.738830.

Sánchez, J. L. F., Le Breton, A., Brun, E., Vendramin, N., Spiliopoulos, G., Furones, D., & Basurco, B. (2022). Assessing the economic impact of diseases in Mediterranean grow-out farms culturing European sea bass. Aquaculture, 547, 737530. https://doi.org/10.1016/j.aquaculture.2021.737530.

Schreck, C. B., & Tort, L. (2016). The concept of stress in fish. In Fish Physiology (Vol. 35, pp. 1-34). Academic Press.

Seafish. (2023). European Sea Bass - *Dicentrachus labrax*. https://www.seafish.org/responsible-sourcing/aquaculture-farming-seafood/species-farmed-in-aquaculture/aquaculture-profiles/european-sea-bass/.

Searchinger, T., Waite, R., Hanson, C., Ranganathan, J., Dumas, P., & Matthews, E. (2019). World resources report: Creating a sustainable food future. World Resources Institute.

Seguin, P., Herault, M., Lee, K. J., & Utairungsee, T. (2018). Functional hydrolysates: sustainable ingredients to support development of the next generation of aquafeeds. AQUA Culture Asia Pacific Magazine January/February 2018.

Shark Research Institute. (2023). Blue shark - *Prionace glauca*. https://www.sharks.org/blue-shark-prionace-glauca.

Sherman, P. K. (2022). Sustainable production of fish protein hydrolysates: Overall system architecture and footprint (Master thesis, Norwegian University of Science and Technology, Norway).

Siddik, M. A., Howieson, J., & Fotedar, R. (2019). Beneficial effects of tuna hydrolysate in poultry by-product meal diets on growth, immune response, intestinal health and disease resistance to *Vibrio harveyi* in juvenile barramundi, *Lates calcarifer*. Fish and Shellfish Immunology, 89, 61-70. https://doi.org/10.1016/j.fsi.2019.03.042.

Siddik, M. A., Howieson, J., Fotedar, R., & Partridge, G. J. (2021a). Enzymatic fish protein hydrolysates in finfish aquaculture: a review. Reviews in Aquaculture, 13(1), 406-430. https://doi.org/10.1111/raq.12481.

Siddik, M. A., Howieson, J., Partridge, G. J., Fotedar, R., & Gholipourkanani, H. (2018). Dietary tuna hydrolysate modulates growth performance, immune response, intestinal morphology and resistance to *Streptococcus iniae* in juvenile barramundi, *Lates calcarifer*. Scientific Reports, 8(1), 1-13. https://doi.org/10.1038/s41598-018-34182-4.

Siddik, M. A., Pham, H. D., Francis, D. S., Vo, B. V., & Shahjahan, M. (2021b). Dietary supplementation of fish protein hydrolysate in high plant protein diets modulates growth, liver and kidney health, and immunity of barramundi (*Lates calcarifer*). Aquaculture Nutrition, 27, 86-98. https://doi.org/10.1111/anu.13404.

Siddiqui, S. A., Schulte, H., Pleissner, D., Schönfelder, S., Kvangarsnes, K., Dauksas, E., Rustad, T., Cropotova, J., Heinz, V., & Smetana, S. (2023). Transformation of seafood side-streams and residuals into valuable products. Foods, 12(2), 422. https://doi.org/10.3390/foods12020422.

Soller, F., Seguin, P., Herault, M., Fournier, V., Trieu, N. V., & Hoa, T. T. T. (2021). Improvement in growth and health performance of juvenile striped catfish with the supplementation of functional protein hydrolysates. AQUA Culture Asia Pacific Magazine July/August 2021.

Sommerset, I., Krossøy, B., Biering, E., & Frost, P. (2005). Vaccines for fish in aquaculture. Expert Review of Vaccines, 4(1), 89-101. https://doi.org/10.1586/14760584.4.1.89.

Spinos, E., Kokkoris, G. D., & Bakopoulos, V. (2017). Prevention of sea bass (*Dicentrarchus labrax*, L. 1758) photobacteriosis and vibriosis. Long term efficacy study of intraperitoneally administered bivalent commercial vaccines. Aquaculture, 471, 172-184. https://doi.org/10.1016/j.aquaculture.2017.01.017.

Sudheesh, P. S., Al-Ghabshi, A., Al-Mazrooei, N., & Al-Habsi, S. (2012). Comparative pathogenomics of bacteria causing infectious diseases in fish. International Journal of Evolutionary Biology, 2012. https://doi.org/10.1155/2012/457264.

Suma, A. Y., Nandi, S. K., Abdul Kari, Z., Goh, K. W., Wei, L. S., Tahiluddin, A. B., Seguin, P., Herault, M., Mamun, A. A., Téllez-Isaías, G., & Anamul Kabir, M. (2023). Beneficial effects of graded levels of fish protein hydrolysate (FPH) on the growth performance, blood biochemistry, liver and intestinal health, economics efficiency, and disease resistance to *Aeromonas hydrophila* of Pabda (*Ompok pabda*) Fingerling. Fishes, 8(3), 147. https://doi.org/10.3390/fishes8030147.

Sun, X., Sarteshnizi, R. A., Boachie, R. T., Okagu, O. D., Abioye, R. O., Neves, R. P., Ohanenye, I. C., & Udenigwe, C. C. (2020). Peptide–mineral complexes: Understanding their chemical interactions, bioavailability, and potential application in mitigating micronutrient deficiency. Foods, 9(10), 1402. https://doi.org/10.3390/foods9101402.

Sunyer, J. O., & Tort, L. (1995). Natural hemolytic and bactericidal activities of sea bream *Sparus aurata* serum are effected by the alternative complement pathway. Veterinary Immunology and Immunopathology, 45(3-4), 333-345. https://doi.org/10.1016/0165-2427(94)05430-Z.

Swanepoel, J. C., & Goosen, N. J. (2018). Evaluation of fish protein hydrolysates in juvenile African catfish (*Clarias gariepinus*) diets. Aquaculture, 496, 262-269. https://doi.org/10.1016/j.aquaculture.2018.06.084.

Tacon, A. G., & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture, 285(1-4), 146-158. https://doi.org/10.1016/j.aquaculture.2008.08.015.

Tang, H. G., Wu, T. X., Zhao, Z. Y., & Pan, X. D. (2008). Effects of fish protein hydrolysate on growth performance and humoral immune response in large yellow croaker (*Pseudosciaena crocea* R.). Journal of Zhejiang University Science B, 9(9), 684-690. https://doi.org/10.1631/jzus.B0820088.

Thiansilakul, Y., Benjakul, S., & Shahidi, F. (2007). Compositions, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (*Decapterus maruadsi*). Food Chemistry, 103(4), 1385-1394. https://doi.org/10.1016/j.foodchem.2006.10.055.

Toranzo, A. E., Barreiro, S., Casal, J. F., Figueras, A., Magariños, B., & Barja, J. L. (1991). Pasteurellosis in cultured gilthead seabream (*Sparus aurata*): first report in Spain. Aquaculture, 99(1-2), 1-15. https://doi.org/10.1016/0044-8486(91)90284-E.

Torrecillas, S., Caballero, M. J., Mompel, D., Montero, D., Zamorano, M. J., Robaina, L., Rivero-Ramírez, F., Karalazos, V., Kaushik, S., & Izquierdo, M. (2017). Disease resistance and response against *Vibrio anguillarum* intestinal infection in European seabass (*Dicentrarchus labrax*) fed low fish meal and fish oil diets. Fish and Shellfish Immunology, 67, 302-311. https://doi.org/10.1016/j.fsi.2017.06.022.

Tort, L. (2011). Stress and immune modulation in fish. Developmental and Comparative Immunology, 35(12), 1366-1375. https://doi.org/10.1016/j.dci.2011.07.002.

Ullah-Khan, K., Tellechea-Rodrigues, A., Menegasso-Mansano, C. F., Queiroz, D. M. D. A., Kazue-Sakomura, N., Romaneli, R. D. S., Nascimento, T. M. T. N., & Kochenborger-Fernandes, J. B. (2019). Dietary protein quality and proper protein to energy ratios: a bioeconomic approach in aquaculture feeding practices. Latin American Journal of Aquatic Research, 47(2), 232-239. https://doi.org/10.3856/vol47-issue2-fulltext-3.

United Nations. (2022). World population prospects 2022: Summary of results. UN DESA/POP/2022/TR/NO. 3.

Varvarigos P. (2020). 10. *Photobacterium damsela* subsp. *piscicida*. In Diagnostic Manual for the Main Pathogens in European Seabass and Gilthead Seabream Aquaculture. Zaragoza : CIHEAM, 2020. p. 83-96 (Options Méditerranéennes : Série B. Etudes et Recherches; n. 75).

Velasco, C., Resende, D., Oliveira, B., Pereira, M., Pereira, C., Pintado, M., & Valente, L. M. (2023). Dietary inclusion of blood hydrolysates affects muscle growth in European seabass (*Dicentrarchus labrax*). Frontiers in Marine Science, 10, 1193405. https://doi.org/10.3389/fmars.2023.1193405.

Villamil, O., Váquiro, H., & Solanilla, J. F. (2017). Fish viscera protein hydrolysates: Production, potential applications and functional and bioactive properties. Food Chemistry, 224, 160-171. https://doi.org/10.1016/j.foodchem.2016.12.057.

Waite, R., Beveridge, M., Brummett, R., Castine, S., Chaiyawannakarn, N., Kaushik, S., Mungkung, R., Nawapakpilai, S., & Phillips, M. (2014). Improving productivity and environmental performance of aquaculture. WorldFish.

Wei, Y., Wang, J., Zhang, X., Duan, M., Jia, L., Xu, H., Liang, M., & Liu, J. (2021). Fish protein hydrolysate supplementation in plant protein based diets for tiger puffer (*Takifugu rubripes*) is an effective strategy of fish meal sparing. Aquaculture Reports, 20, 100720. https://doi.org/10.1016/j.aqrep.2021.100720.

Wendelaar Bonga, S. E. (1997). The stress response in fish. Physiological Reviews, 77(3), 591-625. https://doi.org/10.1152/physrev.1997.77.3.591.

Wu, D., Zhou, L., Gao, M., Wang, M., Wang, B., He, J., Luo, Q., Ye, Y., Cai, C., Wu, P., Zhang, Y., & Pu, Q. (2018). Effects of stickwater hydrolysates on growth performance for yellow catfish (*Pelteobagrus fulvidraco*). Aquaculture, 488, 161-173. https://doi.org/10.1016/j.aquaculture.2018.01.031.

Xu, H., Mu, Y., Zhang, Y., Li, J., Liang, M., Zheng, K., & Wei, Y. (2016). Graded levels of fish protein hydrolysate in high plant diets for turbot (*Scophthalmus maximus*): effects on growth performance and lipid accumulation. Aquaculture, 454, 140-147. https://doi.org/10.1016/j.aquaculture.2015.12.006.

Xu, M., Wang, T., Wang, J., Wan, W., Wang, Z., Guan, D., & Sun, H. (2019). An evaluation of mixed plant protein in the diet of Yellow River carp (*Cyprinus carpio*): growth, body composition, biochemical parameters, and growth hormone/insulin-like growth factor 1. Fish Physiology and Biochemistry, 45, 1331-1342. https://doi.org/10.1007/s10695-019-00641-6.

Zamora-Sillero, J., Gharsallaoui, A., & Prentice, C. (2018). Peptides from fish by-product protein hydrolysates and its functional properties: An overview. Marine Biotechnology, 20, 118-130. https://doi.org/10.1007/s10126-018-9799-3.

Zheng, K., Liang, M., Yao, H., Wang, J., & Chang, Q. (2012). Effect of dietary fish protein hydrolysate on growth, feed utilization and IGF-I levels of Japanese flounder (*Paralichthys olivaceus*). Aquaculture Nutrition, 18(3), 297-303. https://doi.org/10.1111/j.1365-2095.2011.00896.x.

Zheng, K., Liang, M., Yao, H., Wang, J., & Chang, Q. (2013). Effect of size- fractionated fish protein hydrolysate on growth and feed utilization of turbot (*Scophthalmus maximus* L.). Aquaculture Research, 44(6), 895-902. https://doi.org/10.1111/j.1365-2109.2012.03094.x.

Zorrilla, I., Balebona, M. C., Moriñigo, M. A., Sarasquete, C., & Borrego, J. J. (1999). Isolation and characterization of the causative agent of pasteurellosis, *Photobacterium damsela* ssp. *piscicida*, from sole, *Solea senegalensis* (Kaup). Journal of Fish Diseases, 22(3), 167-172. https://doi.org/10.1046/j.1365-2761.1999.00157.x.