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DOUTORAMENTO CIÊNCIA ANIMAL

ESPECIALIDADE EM NUTRIÇÃO

“Farming for You” - Increasing consumer awareness of farmed fish

Daniela Resende

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Instituto de Ciências Biomédicas Abel Salazar

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Tese de candidatura ao grau de Doutor em Ciência Animal, Especialidade em Nutrição (SANFEED), submetida ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto

Instituições de Acolhimento – Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto; Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto; Centro de Investigação em Produção Agroalimentar Sustentável (GreenUPorto); Centro de Biotecnologia e Química Fina (CBQF), Universidade Católica Portuguesa; Sense Test, Lda.

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“Farming for You” - Increasing consumer awareness of farmed fish

Thesis for applying to a Doctor degree in Animal Science, Specialization in Nutrition (SANFEED), submitted to the School of Medicine and Biomedical Sciences (ICBAS) of the University of Porto

Hosting Institutions – Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto; School of Medicine and Biomedical Sciences (ICBAS), University of Porto; Sustainable Agrifood Production Research Center (GreenUPorto); Centro de Biotecnologia e Química Fina (CBQF), Universidade Católica Portuguesa; Sense Test, Lda.

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“I prepared myself for a multitude of reverses; my operations might be incessantly baffled, and at last my work be imperfect, yet when I considered the improvement which every day takes place in science and mechanics, I was encouraged to hope my present attempts would at least lay the foundations of future success.”

- Mary Shelley, *Frankenstein*

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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

Resende, D., Pereira, M.J., Sá, T., Brazinha, C., Pintado, M., Valente, L.M.P., Velasco, C. (2024). Production of aroma-rich extracts from sardine cooking wastewaters: Exploring their potential for modulating feed intake in European seabass. *Waste and Biomass Valorization.* https://doi.org/10.1007/s12649-024-02470-y

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ABSTRACT

The main goal of this thesis is to contribute to the sustainable growth of aquaculture through a holistic approach. The focus is not only on understanding consumer preferences for aquaculture products but also on promoting growth while minimizing economic and environmental impacts. The project aims to achieve this by incorporating sustainable ingredients into fish diets, following a circular economy approach and a "zero waste" policy.

A literature review was conducted, following the PRISMA methodology, to understand the main drivers behind consumption of aquaculture products and how consumers perceive them compared to their wild counterparts. This review underscored that the perception of wild-caught fish as a superior, more natural, and healthier product persists across various geographical backgrounds. Concerns about farmed fish, including the perceived high concentration of chemicals like antibiotics or hormones, continue to impede its acceptance. Consumers generally exhibit low objective and subjective knowledge about aquaculture practices, standards, and products. Additionally, beyond the production method, the country of origin is a crucial factor for consumer choices. Finally, there is an increasing interest in eco-friendly, sustainable and ethical products. The proposed improvements for aquaculture perception include minimizing the use of harmful chemicals, ensuring consumer confidence in this regard. Emphasis should be placed on adopting sustainable and eco-friendly farming practices, with clear labelling. Regions should actively promote their domestic aquaculture products. Lastly, there is a need for widespread dissemination of clear and concise information about aquaculture to enhance consumer knowledge.

In this sense, the shift from traditional, but increasingly unsustainable, fishmeal (FM) diets to plant-based ones is a promising strategy for enhancing aquaculture sustainability and the perceived naturalness of farmed fish. However, implementing plant-based diets for carnivorous fish like the European seabass poses challenges. Nutrient digestibility and fish growth are often hindered due to suboptimal amino acid profiles in plant ingredients, along with a compromised feed intake due to reduced palatability. Additionally, immunity and stress resistance in fish may be weakened. Finally, organoleptic characteristics of fish fillets may be worsened. To address these issues, we propose supplementing plant-based diets with functional ingredients that offer benefits beyond basic nutrition.

Firstly, we developed hydrolysates from swine blood, a by-product of the meat industry. The hydrolysis allows the breakdown of the blood proteins, releasing bioactive peptides that are encoded in their sequence. Swine blood was processed either by auto-hydrolysis (AH) or enzymatically. The hydrolysates obtained enzymatically were further submitted to a micro- (RMF) and nanofiltration (RNF) and the respective retentates

freeze-dried. Dried hydrolysates were then included in isolipidic and isoproteic diets for European seabass juveniles: a FM based diet (positive control, PC), a diet where 50% of FM was replaced by vegetable proteins (negative control, NC) and three diets where 3% of each hydrolysate was added to the NC.

The PC led to the highest final weight, above that of the NC, supporting the notion that plant-based diets hinder growth. However, digestibility, feed conversion ratio (FCR) and intestinal morphology were similar between the two diets. The hydrolysate with the best growth performance was RNF, which showed no significant differences from the NC on final weight and protein and lipid digestibility. It was even statistically similar to the PC in terms of protein digestibility, FCR, daily growth index (DGI) and intestinal morphology. RMF had the worst growth performance of all diets, along with significant impairments in intestinal development and nutrient digestibility. AH led to a better growth performance than RMF, but it was still inferior to both controls.

After the growth trial, fish were challenged with the pathogenic *Tenacibaculum maritium* to assess whether the hydrolysates had improved the fish's resistance to it. RNF had the lowest mortality rate, being significantly lower compared to the NC. Mortality for fish fed AH and PC did not differ from RNF. RMF showed the highest mortality. Overall, results indicate that dietary supplementation with RNF clearly improves the resistance of European seabass to *T. maritimum* without affecting growth rate and can hence become a valuable ingredient for this species.

The impact of hydrolysates as oxidative stress modulators was also assessed after the growth trial, by exposing the fish to a multifactorial stress challenge: 1 min air exposure followed by 6 h recovery in different tanks. The challenge increased fish circulating cortisol and liver lipid peroxidation (LPO) due to reactive oxygen species (ROS) accumulation. None of the hydrolysates was able to reduce either parameters compared to a plant-based control, but diets with the hydrolysates seemed to affect the fish oxidative stress response in the liver, possibly due to the presence of bioactive peptides, which aided in the non-enzymatic modulation of stress response, as observed by the total antioxidant capacity values in the liver.

To mitigate the reduced palatability of plant-based aquafeeds, we proposed supplementing them with extracts from sardine cooking wastewaters, which possess a "fish" odour. These by-products from the canning industry are complex to process, thus presenting an opportunity for valorisation. Sardine cooking wastewaters were collected and tested directly (CW-A) or after vacuum distillation (VD-A) or liquid/liquid extraction with soybean oil (LLE-A). Despite losses in aldehydes and short-chain alcohols, both processes were effective in removing off-flavours. VD-A displayed a higher concentration of most aromas compared to LLE-A. Extracts were included at 2 μg/g of 1-penten-3-ol, the most abundant compound in all extracts, in diets (CW, VD, LLE). A non-supplemented plant-based diet was used as control. After 18 weeks, fish fed LLE displayed a significantly higher feed intake than CW, although all dietary treatments were like the control. The increase in feed intake was correlated with a higher neuropeptide Y (*npy*) expression in the hypothalamus. Liver and plasma metabolite analysis, however, suggests that LLE slightly hinders lipid metabolism, and thus the increase in feed intake could have been due to a combination of homeostatic (driven by metabolic nutrient or energy needs) and hedonic (pleasure-driven) regulation of feed intake. To further assess this, a short-term trial was performed, feeding fish a single meal until apparent satiation. Our emphasis was on this initial feeding to comprehend the hedonic control of feed intake. Feed intake was highest for the control group. No differences on plasma metabolites were observed, suggesting feed intake was primarily regulated by hedonic rather than homeostatic mechanisms. Moreover, the lower intake in the supplemented diets was partially associated with a lower expression of orexigenic (intake-promoting) neuropeptides and higher expression of anorexigenic (intake-reducing) neuropeptides in the brain, despite the lack of significant diet-related differences. Overall, it is possible that the aroma extracts hinder the hedonic regulation of feed intake, but at the longer-term, animals display habituation and their intake is more heavily influenced by nutritional needs.

In parallel, we also performed a sensory analysis of the fish fed the aroma-supplemented diets. This evaluation was deemed necessary due to the potential for flavour modulation resulting from the aromas. The concern arose from the possibility of altered fish metabolism, as observed with the LLE diet, which could affect the texture and flavour of the fillet. Previous research has also indicated that plant-based diets may have negative effects on the texture, skin colour, and flavour of European seabass. We performed a sensory analysis with a consumer panel (n=70) at the Sense Test facilities, using the Rate-All-That-Apply (RATA) methodology. All diets had a similar sensory profile and an average overall liking equal or above to 7.5 in a 1 to 9 scale. This is particularly important because the dietary treatments had high levels of plant-based ingredients, which did not seem to compromise overall liking. Moreover, the use of different aromas, regardless of their impact on fish growth or nutrient utilisation, did not affect the sensory profile of European seabass by the consumer panel, even when a highly discriminative methodology such as RATA is employed.

RESUMO

O principal objetivo desta tese é contribuir para o crescimento sustentável da aquacultura através de uma abordagem holística. O foco está na compreensão das preferências dos consumidores em relação aos produtos de aquacultura, mas também na promoção do crescimento do sector, minimizando simultaneamente os impactos económicos e ambientais. Pretende-se concretizar isto através da incorporação de ingredientes sustentáveis nas dietas dos peixes, seguindo uma abordagem de economia circular e uma política de "desperdício zero".

Realizou-se uma revisão da literatura, seguindo a metodologia PRISMA, para compreender como os consumidores percecionam o peixe de aquacultura em comparação com o peixe selvagem. Foi observado que a perceção do peixe selvagem como um produto superior, mais natural e mais saudável, persiste em vários contextos geográficos. As preocupações com os peixes de aquacultura, incluindo a possível presença de químicos como antibióticos ou hormonas, continuam a dificultar a sua aceitação. Genericamente, os consumidores apresentam baixo conhecimento sobre as práticas e produtos de aquacultura. Para além do método de produção, o país de origem é um fator crucial para a escolha dos consumidores. Por fim, há um interesse crescente em produtos ecológicos, sustentáveis e éticos. Para melhorar a perceção da aquacultura, sugere-se minimizar o uso de produtos químicos, e garantir isso aos consumidores. Deverão enfatizar-se práticas aquícolas sustentáveis e ecológicas, com rotulagem clara. Cada região deverá promover ativamente os seus produtos de aquacultura. Por último, é necessária uma ampla divulgação de informação clara e concisa sobre a aquacultura para aumentar o conhecimento sobre a área.

Neste sentido, a mudança de dietas tradicionais, mas cada vez mais insustentáveis, à base de farinha de peixe (FM) para dietas vegetais é uma estratégia promissora para melhorar a sustentabilidade da aquacultura e a perceção da naturalidade dos peixes de aquacultura. Porém, o uso de dietas vegetais para peixes carnívoros como o robalo é desafiante. A digestibilidade dos nutrientes e o crescimento dos peixes são frequentemente prejudicados devido a piores perfis de aminoácidos nos ingredientes vegetais, juntamente com um menor consumo de ração devido à reduzida palatabilidade. Além disso, a imunidade e a resistência ao stress externo dos peixes podem ser enfraquecidas. As características organoléticas dos produtos finais podem ainda ser prejudicadas. Para contrariar isto, propôs-se complementar as dietas vegetais com ingredientes funcionais que oferecem benefícios para lá da nutrição básica.

Primeiramente, desenvolveram-se hidrolisados de sangue suíno, subproduto da indústria da carne. A hidrólise permite a quebra das suas proteínas, libertando péptidos

bioativos que estão codificados na sua sequência. O sangue suíno foi processado por auto-hidrólise (AH) ou enzimaticamente. Os hidrolisados obtidos enzimaticamente foram depois submetidos a micro (RMF) e nanofiltração (RNF) e os respetivos retentados liofilizados. Os hidrolisados foram então incluídos em dietas isolipídicas e isoproteicas para juvenis de robalo: uma dieta rica em FM (controlo positivo, PC), uma dieta onde 50% da FM foi substituída por proteínas vegetais (controlo negativo, NC) e três dietas onde 3% de cada hidrolisado foi adicionado ao NC.

O PC levou ao maior peso final, acima do NC, confirmando que as dietas vegetais dificultam o crescimento. A digestibilidade, a taxa de conversão alimentar (FCR) e a morfologia intestinal foram semelhantes entre as duas dietas. O hidrolisado com melhor desempenho foi o RNF, que não apresentou diferenças significativas para o NC no peso final e na digestibilidade proteica e lipídica. Foi ainda estatisticamente idêntico ao PC em termos de digestibilidade proteica, FCR, índice de crescimento diário e morfologia intestinal. O RMF teve o pior desempenho de todas as dietas, juntamente com danos significativos no desenvolvimento intestinal e na digestibilidade. AH levou a um melhor desempenho de crescimento do que RMF, mas ainda foi inferior a ambos os controlos.

Após o ensaio de crescimento, os peixes foram desafiados com a bactéria *Tenacibaculum maritium*, para avaliar se os hidrolisados melhoravam a imunidade dos peixes. O RNF apresentou a menor mortalidade, sendo significativamente menor em comparação ao NC. A mortalidade dos grupos AH e PC não diferiu do RNF. RMF teve a maior mortalidade. Globalmente, a suplementação das dietas com RNF melhora a resistência do robalo a *T. maritimum* sem afetar o seu crescimento e pode, assim, ser um ingrediente valioso para esta espécie.

Avaliou-se ainda o potencial dos hidrolisados como moduladores do stress oxidativo após o ensaio de crescimento, expondo os peixes a um desafio multifatorial: 1 min de exposição ao ar seguido de 6 h de recuperação em tanques diferentes. O desafio aumentou o cortisol plasmático e a peroxidação lipídica do fígado devido à acumulação de espécies reativas de oxigénio. Nenhum dos hidrolisados foi capaz de reduzir estes parâmetros em comparação com o controlo vegetal, mas as dietas com hidrolisados afetaram a resposta ao stress oxidativo ao nível do fígado, talvez devido à presença de péptidos bioativos, que auxiliaram na sua modulação não enzimática, conforme observado pelos valores da capacidade antioxidante total no fígado.

Para minimizar os problemas de palatabilidade das dietas vegetais, propusemos a sua suplementação com extratos de águas residuais da cozedura da sardinha com um odor a “peixe”. Trata-se de um subproduto da indústria conserveira que necessita de um tratamento complexo, pelo que seria assim valorizado. As águas de cozedura foram recolhidas e testadas diretamente (CW-A) ou após destilação a vácuo (VD-A) ou extração líquido/líquido com óleo de soja (LLE-A). Apesar das perdas em aldeídos e álcoois de cadeia curta, ambos os processos foram eficazes na remoção de “off-flavours”. O VD-A apresentou maior concentração da maioria dos aromas em comparação ao LLE-A. Os extratos foram incluídos nas dietas a 2 μg/g de 1-penten-3-ol, composto mais abundante em todos os extratos (CW, VD, LLE). O controlo foi uma dieta vegetal não suplementada. Após 18 semanas, os peixes alimentados com LLE ingeriram significativamente mais ração do que o CW, embora todos os tratamentos tenham sido semelhantes ao controlo. O aumento da ingestão correlacionou-se com uma maior expressão do neuropéptido Y (*npy*) no hipotálamo. A análise dos metabolitos do fígado e do plasma, contudo, sugere que o LLE prejudica o metabolismo lipídico, e esse aumento no consumo pode dever-se a uma combinação entre a regulação homeostática (impulsionada pelas necessidades de nutrientes ou energia) e hedónica (impulsionada pelo prazer). Para uma melhor avaliação, realizou-se um ensaio curto, alimentando os peixes com uma única refeição até alcançarem saciedade aparente. A ênfase foi dada a essa alimentação inicial para compreender o controlo hedónico. O consumo de ração foi maior no grupo controlo. Não existiram diferenças nos metabolitos plasmáticos, sugerindo que a ingestão foi regulada principalmente por mecanismos hedónicos e não homeostáticos. Além disso, a menor ingestão nas dietas suplementadas foi parcialmente associada a uma menor expressão de neuropéptidos orexigénicos (promotores da ingestão) e a uma maior expressão de neuropéptidos anorexigénicos (redutores da ingestão) no cérebro, apesar da ausência de diferenças significativas entre dietas. No geral, é possível que os extratos com aromas prejudiquem a regulação hedónica da ingestão, mas a longo prazo, os animais apresentem habituação, sendo a ingestão mais fortemente influenciada pelas necessidades nutricionais.

Paralelamente, realizou-se ainda uma análise sensorial dos peixes alimentados com dietas suplementadas com aromas, devido ao potencial de modulação do sabor pelos mesmos, e à possibilidade de alteração do metabolismo dos peixes (como observado na dieta LLE) capazes de afetar a sua textura e o sabor. Realizamos então uma análise sensorial com um painel de consumidores (n=70) nas instalações da Sense Test, utilizando a metodologia “Rate-All-That-Apply” (RATA). Todas as dietas apresentaram perfil sensorial semelhante e uma aceitação média igual ou superior a 7,5 numa escala de 1 a 9. Isto é particularmente importante porque as dietas continham elevados níveis de ingredientes vegetais, o que não pareceu comprometer a sua aceitação. Além disso, a utilização de diferentes aromas, independentemente do seu impacto no crescimento dos peixes ou na utilização de nutrientes, não afetou o perfil sensorial do robalo pelo painel de consumidores, mesmo quando utilizando uma metodologia altamente discriminativa como o RATA.

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AA	Amino acid(s)
AB-PAS	Alcian-blue/periodic acid Schiff
ABTS	2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid
ABW	Average body weight
ACH50	Alternative complement pathway
ADC	Apparent digestibility coefficient
afmid	Arylformamidase-like
agrp	Agouti-related peptide
AH-H	Hydrolysate by autohydrolysis
AH	Diet with 3% hydrolysate by autohydrolysis
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
AP	Alkaline phosphatase
ASC	Aquaculture Stewardship Council
β-act	Beta-actin
BHT	Butylated hydroxytoluene
BH	Blood hydrolysates
BL	Body length
BP	Bioactive peptide(s)
BW	Body weight
CAGR	Compound annual growth rate
capn1	Calpain 1
CAR/PDMS	Carboxen/Polydimethylsiloxane
cart	Cocaine and amphetamine-related transcript
casp-3	Caspase-3
CATA	Check all that apply
CAT	Catalase
CC	Carbonyl compounds
cd3ζ	Cluster of differentiation 3 ζ chain
cd4	Cluster of differentiation 4
cd8α	Cluster of differentiation 8 α chain
cd8β	Cluster of differentiation 8 β chain
cdh17	Cadherin-17
cDNA	Complementary DNA

CDNB	1-chloro-dinitrobenzene
CE	Circular economy
CF	Crude fat
cfu	Colony forming units
COO	Country of origin
cox2	Cyclooxigenase-2
CP	Crude protein
CTRL	Control
CV	Coefficient of variation
CW-A	Aromas from cooking wastewaters
CW	Diet with cooking wastewaters
cxcr4	Chemokine cxc receptor 4
cyp11b	Cytochrome P450 family 11 subfamily b
DCE	Discrete choice experiment
DCP	Di-calcium phosphate
DEI	Digestible energy intake
DGI	Daily growth index
DHA	Docosahexaenoic acid
DLI	Digestible lipid intake
DM	Dry matter
DNI	Digestible nitrogen intake
DP/DE	Digestible protein/digestible energy ratio
DPI	Digestible phosphorus intake
DPPH	1,1-diphenyl-2-picrylhydrazyl
DTNB	5,5’-dithiobis-2-nitrobenzoic acid
DTPA	Diethylenetriaminepentaacetic acid
EAA	Essential amino acids
EDTA	Ethylenediaminetetraacetic acid
ef1α	Elongation factor 1 alpha
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
ERE	Energy retention efficiency
EUMOFA	European Market Observatory for Fisheries and Aquaculture Products
EU	Enzyme unit
FAO	Food and Agriculture Organization of the United Nations

FAS	Fatty acid synthetase
FCR	Feed conversion ratio
FDA	Food and Drug Administration
FF	Farmed fish
fgf4	Fibroblast growth factor 4
fgf6	Fibroblast growth factor 6
FM	Fishmeal
FO	Fish oil
FPLC	Fast protein liquid chromatography
G6PD	Glucose-6-phosphate dehydrogenase
GC-MS	Gas chromatography - mass spectrometry
GPx	Glutathione peroxidase
gr-1	Glucocorticoid receptor 1
gr-2	Glucocorticoid receptor 2
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione S-transferase
h1f-1α	hypoxia inducible factor
HACCP	Hazard analysis and critical control points
HDL	High-density lipoprotein
HSI	Hepatosomatic index
hsp70	Heat shock protein 70
ICP-OES	Inductively coupled plasma-optical emission spectrometry
ido2	Indoleamine dioxygenase 2
igf1	Insulin-like growth factor 1
IgM	Immunoglobulin M
il1β	Interleukin 1 β
il6	Interleukin 6
il8	Interleukin 8
il10	Interleukin 10
IMTA	Integrated multi-trophic aquaculture
K	Condition index
lap	Leucine aminopeptidase N
LC-PUFA	Long chain polyunsaturated fatty acid(s)
LER	Lipid efficiency ratio

leu-ala	Leucine – alanine peptidase
LLE-A	Aromas from liquid-liquid extraction
LLE	Diet with liquid-liquid extraction aromas
LPO	Lipid peroxidation
LPS	Lipopolysaccharide
LRE	Lipid retention efficiency
MB	Marine broth
mc2r	Melanocortin 2 receptor
MCV	Mean corpuscular volume
MDA	Malondialdehyde
ME	Malic enzyme
MF	Microfiltration
mhcII	Major histocompatibility complex II
mmp9	Matrix metalloproteinase
mRNA	Messenger ribonucleic acid
MSC	Marine Stewardship Council
mTor	Mechanistic target of rampamycin
mx	mx protein
mymk	myomaker
myod1	myoblast determination factor
NADPH	Nicotinamide adenine dinucleotide phosphate
NC	Negative control
NEAA	Non-essential amino acids
NEFA	Non-esterified fatty acid(s)
NO	Nitric oxide
noxin	Nitric oxide inducible gene protein
npy	Neuropeptide Y
NRE	Nitrogen retention efficiency
OD	Optical density
ORAC	Oxygen radical absorbance capacity
PC	Positive control
PCA	Principal component analysis
pcna	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PER	Protein efficiency ratio
Phdp	*Photobacterium damselae* subsp. *piscicida*

pomc	Pro-opiomelanocortin
PRE	Phosphorus retention efficiency
PRISMA	Preferred reporting items for systematic reviews and meta analyses
PUFA	Polyunsaturated fatty acids
RAS	Recirculating water system
RATA	Rate all that apply
RMF-H	Retentate from microfiltration
RMF	Diet with 3% retentate from microfiltration
RNA	Ribonucleic acid
RNF-H	Retentate from nanofiltration
RNF	Diet with 3% retentate from nanofiltration
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rps40	Ribosomal protein 40S
sat1	Spermine/spermidine N (1)-acetyltransferase
SGR	Specific growth rate
slc6a16a	Solute carrier family 6 member 16a
slc38a2	Solute carrier family 38 member a2
SOD	Superoxide dismutase
SPME	Solid phase microextraction
STaR	steroidogenic acute regulatory protein
T1R	Taste receptor type 1
T2R	Taste receptor type 2
TAC	Total antioxidant capacity
TBARS	Thiobarbituric acid reactive substances
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
tcrα	T-cell receptor α chain
TE	Trolox equivalents
TG	Total glutathione
tgfβ	Transforming growth factor β
tnfα	Tumor necrosis factor α
TNB	5-thio-2-nitrobenzoic acid
UPLC	Ultra-high-performance liquid chromatography
VD-A	Aromas from vacuum distillation
VD	Diet with aromas from vacuum distillation

VFI	Voluntary feed intake
VSI	Viscerosomatic index
WBC	Whole-body composition
WF	Wild fish
WTP	Willingness to pay

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1. INTRODUCTION

1.1. Aquaculture

Aquaculture is defined by the European Union (EU) as “the rearing or cultivation of aquatic organisms using techniques designed to increase the production of the organisms in question beyond the natural capacity of the environment” (Council of the European Union, 2006). In 2021, the EU aquaculture production reached 1.13 million tonnes, which can be translated to a value of € 4.17 billion (EUMOFA, 2023b), showcasing the great importance of this sector to the EU economy. Aquaculture is an area with increasing demand, posing on researchers the challenge to continuously improve and overcome the obstacles associated with this industry. It is worth noting that 2020 values are lower than those reported in previous years, mainly due to the COVID-19 pandemic. In particular, this affected sales to the hotel, restaurant and catering businesses. However, there was an increase in 11% in the aquaculture production from 2012 to 2021 (EUMOFA, 2023b).

The industrial farming of European seabass (*Dicentrarchus labrax*) began in the late 1980s and has been steadily growing, even though 10% production still originates from fishing (Llorente et al., 2020). As of 2021, seabass accounted for 15% of European Union aquaculture production, in terms of value, (Figure 1, left), which can be translated into a €590 million impact on the EU economy (EUMOFA, 2023b). The main EU producers of this species are located in the Mediterranean region, and include Greece, Spain, Italy and Portugal. In Portugal (Figure 1, right), seabass aquaculture production accounts for 5% (€7.3 million) of the total aquaculture value (EUMOFA, 2023a). This species is mostly marketed as whole and fresh, and generally consumers show a positive perception of European seabass in terms of its health and nutritional properties, quality, environmental impact and price (Vanhonacker et al., 2013).

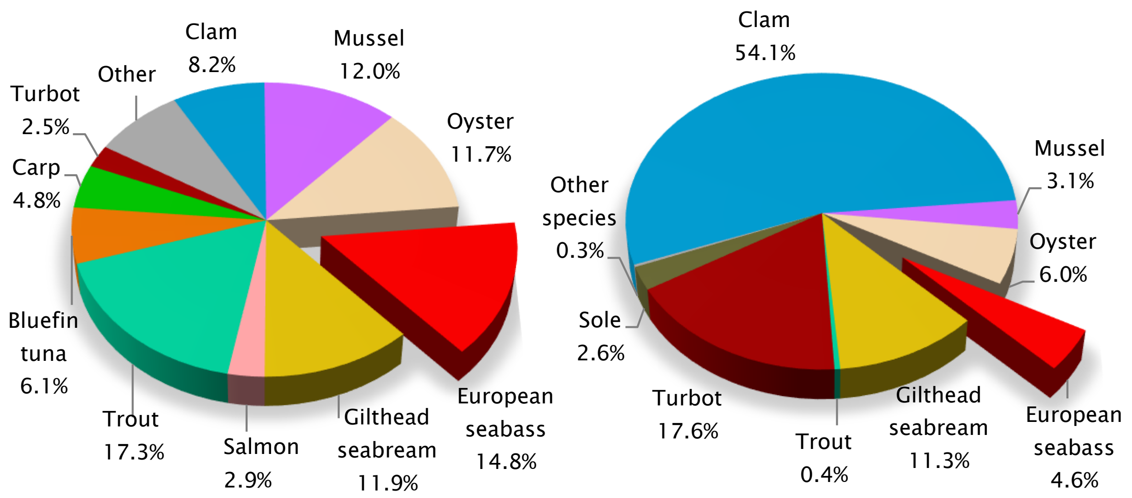

Figure 1 - Aquaculture production (in value), by species: on the left, European Union production; on the right, Portugal production, as of 2020.

1.2. Aquafeeds

One of the most important factors in the success of semi-intensive and intensive aquaculture businesses is the feed (Naylor et al., 2021; Sun et al., 2016). Indeed, fed aquaculture production has been outpacing the farming of non-fed species, achieving a share of 72.2% according to the latest FAO data (FAO, 2022). In the development of feeds, several factors are essential, including the species nutritional requirements, production costs and sustainability of ingredients.

1.2.1. European seabass nutritional requirements

European seabass is a carnivorous fish. This implicates that their feed must contain high levels of protein, which vary with age and weight. For fish below 20 g, 55% is the recommended dietary protein level; for fish above 200 g, this value decreases to 45% (National Research Council, 2011). Furthermore, the ratio between total digestible protein and digestible energy (DP/DE) is vital for optimal growth and nutrient utilization efficiency, while also having an economical implication: an excess of protein in diets can be wasted or used as an energy source rather than being deposited. On the other hand, using an excess of non-protein dietary energy can hinder food intake and growth and promote adipose tissue growth, thus affecting final product quality (Kousoulaki et al., 2015). The optimal DP/DE is weight-dependant and can range from 20 to 25 mg protein kJ^{-1} (Kousoulaki et al., 2015). Importantly, beyond the overall protein content in a diet, the crucial factor lies in its capacity to fulfil the animal's specific amino acid (AA) needs.

Dietary AA balance is critical to ensure optimal growth, with seabass requiring 10 essential AA: arginine, histidine, lysine, threonine, isoleucine, leucine, valine, phenylalanine, methionine and tryptophan (National Research Council, 2011). Nevertheless, a balanced ratio between essential and non-essential AA is also crucial, and this ratio has been suggested to be 50/50 or 60/40 (Peres & Oliva-Teles, 2006). Therefore, in the formulation of aquafeeds, this must be taken into account when choosing the main protein sources, and if necessary, supplementation with crystalline AA should be done to ensure that the dietary needs are fulfilled.

Historically, protein is the most expensive macronutrient of aquafeeds. As such, aquafeeds are commonly formulated to favour other nutrients, such as lipid or carbohydrates for energetic catabolism and spare proteins for deposition, an effect known as “protein sparing” (Francis & Turchini, 2017). Although an exact minimum lipid requirement cannot be defined (as this is influenced by a number of nutritional factors), an inclusion level of 12-20% is recommended in order to improve growth rate and protein efficiency of European seabass (National Research Council, 2011). Levels above 30% have been reported to hinder growth performance by decreasing energy and protein retention efficiencies (Peres & Oliva-Teles, 1999). In terms of fatty acids, n-3 long chain polyunsaturated fatty acids (LC-PUFA), such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids are essential, as the European seabass lacks the ability to elongate linoleic and α-linolenic acids into these. Advised levels of EPA and DHA range between 0.7-1% as dry matter basis (National Research Council, 2011; Skalli & Robin, 2004). The DHA:EPA ratio also seems to be important in aquafeeds, but its optimal value is yet to be determined (Tocher, 2010).

Despite reports of high digestibility of dietary carbohydrates (Enes et al., 2011), these do not show necessarily protein or energy sparing effects and may even depress feed intake and utilisation or growth (Kousoulaki et al., 2015; Peres & Oliva-Teles, 2002). Consequently, upper limits of 20% have been established (Enes et al., 2011).

European seabass requirements of vitamins and minerals remain largely unexplored. Water-soluble vitamin levels have been extrapolated from studies in salmonids (Kaushik et al., 1998), while fat-soluble vitamin requirements have been mostly studied in larvae (Kousoulaki et al., 2015). Mineral needs are highly affected by water composition, since saltwater fish get an important fraction of their mineral needs from surrounding water (Lall & Kaushik, 2021), which further complicates the determination of accurate dietary needs. Regarding phosphorus dietary needs, this value has been set at a minimum of 6.5 g kg^{-1} by Oliva-Teles and Pimentel-Rodrigues (2004), for European seabass juveniles. However, P bioavailability is highly dependent on its supplementation source, depending on its organic or inorganic form; the most common inorganic supplement used in aquafeeds is the di-calcium phosphate. Calcium requirements have been poorly explored, despite the importance of this mineral for the development of skeletal tissues (National Research Council, 2011). The same applies to potassium. Concerning microminerals, requirements have not been explored either, despite the importance of some, such as copper, which is a known cofactor of enzymes involved in the oxidative status regulation, or manganese, which participates in fatty acid and amino acid metabolism (Lall, 2003). For iron, while an upper limit of 750 mg kg^{-1} (Maage et al., 2008) has been established by the EU, minimum requirements are unknown. It should be taken into consideration that high levels of iron can also promote feed oxidation, in particular of polyunsaturated fatty acids.

1.2.2. Sustainability in aquafeeds: the challenge of fishmeal replacement

In fish farms, feed can account for 40%-60% of the production costs, as aquafeeds have usually a high protein content, particularly for carnivorous species (Prem & Tewari, 2020). Moreover, feed is responsible for a large portion of waste produced in fish farms (Dauda et al., 2019).Therefore, it is important to use raw materials that have a high digestibility, decreasing the faecal losses and waste produced, and a high palatability, to minimize the amount of uneaten feed.

High quality fishmeal (FM) has often been considered as an optimal protein source for carnivorous fish such as European seabass, since it provides the most adequate AA profile for such species (Peres & Oliva-Teles, 2007). Nevertheless, the continuous growth of the aquafeed sector has led to overfishing to meet FM demands. As a result, this ingredient has become environmentally and economically unstainable (Egerton et al., 2020; Naylor et al., 2021). Currently, to overcome this, one third of global FM comes from trimmings from both wild fisheries and aquaculture, considering a circular economy framework (Naylor et al., 2021). However, as aquaculture keeps growing, this is not enough to supply the protein needs of the sector, highlighting the need for alternative protein sources.

Research on aquafeeds has been targeting the replacement of FM without compromising the quality and potential of the feed. Several alternatives have recently emerged and been evaluated in aquafeeds. Insect meal could be a promising alternative, since insects do not require arable land, and have low needs of water and energy (Basto et al., 2020). Nevertheless, variability of insect meals is very high and often its nutritional profile is not ideal. Additionally, they can contain high levels of chitin, which is anti-nutritional (Hua et al., 2019). Regarding economic considerations, currently, insect meals are not price competitive, as demonstrated by the work of Arru et al. (2019), who have found that the inclusion of *Tenebrio molitor* meal would increase the cost of feeds for European seabass. Microbial biomass, which includes bacteria, yeast and microalgae, is increasingly seen as a potential protein source for aquafeeds. However, and despite their high protein contents and AA profiles that resemble FM's, scalability is still an issue and an economically viable production of these protein sources is still being investigated (Hua et al., 2019). Macroalgae also seem to have ideal AA profiles to many species of carnivorous fish. In spite of this, macroalgae have high levels of dietary fibre, which not only lead to lower absolute concentrations of AA in dry weight, but also hinder the digestibility of feed (Fleurence et al., 2012). As such, currently, algae usage has mostly been considered from a functional point of view due to their bioactive compounds (Wan et al., 2019)

Overall, the most commonly used protein sources to replace FM are plant proteins, such as soybean, corn, barley, peas or wheat (Jannathulla et al., 2019). While, in the past, FM prices tended to be more appealing than some plant-based alternatives, as the scarcity of this ingredient rises, FM prices are also on the rise, and thus plant proteins are now economically viable alternatives (Naylor et al., 2021). Moreover, plant-based proteins are abundantly available in the market, ensuring ample supply. Despite this, plant-based ingredients also pose sustainability issues, namely increased ecotoxicity due to concentration of fertilizers and pesticides, increased pressure on land resources and freshwater, and deforestation leading to biodiversity loss (Malcorps et al., 2019; Pelletier et al., 2018). Furthermore, plant proteins should have low levels of anti-nutritional factors, including non-starch polysaccharides, phytates, protease inhibitors, saponins, among others (Kokou & Fountoulaki, 2018). Antinutrients interfere with other feed compounds' utilisation, affecting fish overall health status and growth. Plant proteins are known to have high concentration of these anti-nutritional factors, but some mitigation methods have been developed, such as chemical or mechanical processing, genetic improvement of plants or the co-inclusion of enzymes able of degrading the antinutrients (Kumar & Barman, 2012; Valente et al., 2021). This has resulted in the development of several vegetable protein concentrates with very high digestibility, such as soy protein concentrate. In parallel, it is important to find plant-based ingredients whose AA profile addresses the species requirements. This is not the case most of the times, and to surpass this issue, complementary plant ingredients can be included in feed formulations or they can be supplemented with crystalline AA (Güroy et al., 2013; Naylor et al., 2009). However, most of the times this is not enough for carnivorous fish, and thus only partial replacement of FM with plant-based ingredients is feasible. Moreover, the supplementation with crystalline AA leads to differences between the profile of absorbed essential AA and that of the diet, diminishing protein efficiency as the

AA in excess will be preferentially catabolized for energy. Coating AA with agar can help balancing absorption rates (Kousoulaki et al., 2015).

Replacing marine ingredients with vegetable alternatives can interfere with the animal's immune system, leading to a lower resistance to possible infections (Krogdahl et al., 2000; Siddik et al., 2021; Torrecillas et al., 2017a). Similarly, there are reports of fish displaying a poorer response to external stressors when high levels of plant-based ingredients are used (Machado et al., 2019; Pelusio et al., 2022).

Finally, palatability is also often limited in diets with high levels of plant proteins, leading to a low intake of the diets (Morais, 2017). Including feed attractants, such as betaine, amino acid mixtures, marine hydrolysates or others, is an alternative to increase diet palatability (Dias et al., 1997; Jiang et al., 2019).

Taking all of this into consideration, the use of plant protein sources is still the most frequently chosen alternative to FM, considering their price and availability. However, further research is still needed to improve welfare along with resistance to stress and infections of fish fed plant-based diets. Moreover, growth performance can also be enhanced, through the increase of diet palatability, which is still a significant issue of plant-based diets. For these reasons, in European seabass juveniles, the FM content currently cannot be lowered below 10% without impairing the animal's growth or health status (Bonvini et al., 2018; Gonçalves et al., 2019). To mitigate these potential negative impacts, supplementing plant-based diets with functional ingredients could be an effective strategy to yield a diet with benefits comparable to those of a FM-based diet.

1.3. Functional ingredients: formulating diets towards challenging conditions

The development of intensive aquaculture is hindered by external stress factors that animals face in these systems. Stress has been defined as: "*The physiological cascade of events that occurs when the organism is attempting to resist death or re-establish homeostatic norms in the face of insult*" (Schreck, 2000; Schreck & Tort, 2016). Examples of these "insults" are: changes in dissolved oxygen, water temperature, nitrogen compounds, salinity; management interferences, including high stocking densities, frequent handling procedures, transportation, confinement; and social factors regarding interactions of fish due to courtship, foraging or social purposes (Li et al., 2022). Such challenges have recently gained importance in a climate change scenario that obliges fish to cope with more frequent stressful events (Maulu et al., 2021).

Stress responses of fish can be classified as primary, secondary or tertiary. The primary response is related to the activation of endocrine pathways in the hypothalamo-sympathetic and the hypothalamus-pituitary-interrenal axes (Barton, 2002). The increase in circulating corticosteroid and cathecolamine hormones, including cortisol, leads to the secondary response, which refers to the metabolic, hydromineral, cardiovascular, respiratory and immune changes that occur in the animal (Schreck & Tort, 2016). Energy substrates are released into circulation, often resulting in raised levels of glucose and lactate, and lowered tissue glycogen, for example (Barton, 2002). There is an increase in cardiac rhythm (Brijs et al., 2018), whilst an hydromineral dysfunction occurs, because adrenaline changes both the gill blood flow patterns and permeability, altering osmotic balance. Immune suppression might also take place (Schreck & Tort, 2016). Moreover, an unbalance between reactive oxygen species (ROS) or reactive nitrogen species (RNS) and radical scavenging by antioxidants (or antioxidant enzymes) might occur, leading to oxidative/nitrosative stress. This can result in lipid peroxidation (LPO), carbonyl compounds (CC) formation (from degradation of proteins) or damage to nucleic acids. Finally, tertiary responses are generally maladaptive, and they comprise alterations in whole-animal performance, including impaired growth, poor health status, behavioural issues and reproduction alterations (Barton, 2002; Sadoul et al., 2021).

It is also worth noting that stress itself may not be detrimental. There are three stages of the stress response, according to Schreck and Tort (2016): alarm, resistance and compensation or exhaustion. The “alarm” comprises the upregulation of mechanisms directly linked to flight, fight and coping responses. Then, the “resistance” phase occurs, during which the fish can fully recover from the stress, overcome the stressor just enough to compensate, or start a downward trajectory that will eventually lead to the animal’s death. Low stress levels can lead to the first scenario, but higher intensity or chronical stress can result in the negative aforementioned outcomes.

In fish farms, including in European seabass farms, infectious diseases are one of the major causes of fish mortality (Galindo-Villegas & Mulero, 2014). Better management practices, such as improved sanitary conditions, clean water supply or vector control, are the most effective ways to prevent disease. In parallel, a deep understanding of each species immune system allows for the development of strategies for its improvement, including vaccination and the supplementation of immunostimulant compounds in aquafeeds. These strategies are particularly important, considering the growing intensification of aquaculture, and the issues posed by climate change.

The seabass immune system possesses elements of both innate (non-specific) and adaptative immunity. However, the production of thymocytes is delayed and the seabass is incapable of synthesizing antibodies until several weeks after hatching. This is compensated by innate mechanisms that are fully functional by hatching (Galindo-Villegas & Mulero, 2014; Swain & Nayak, 2009). These play an essential role in the combat of pathogens and include several mechanisms. Physical obstacles (skin, epithelial tissue lining, mucus) aim at trapping and eliminating the infectious agent directly (Cabillon & Lazado, 2019). Specialized cells (macrophages, granulocytes, among others) also play a vital role in this response. Finally, chemical factors include the pH of body fluids and immune-reactive molecules (such as antimicrobial peptides, lysozyme, agglutinin, precipitins, antibacterial lytic enzymes or transferrin). Antimicrobial peptides are small AA chains that can neutralize a broad range of microbes, including bacteria, fungi, virus or parasites (Galindo-Villegas & Mulero, 2014). Lysozymes are enzymes that can hydrolyse the bond between *N*-acetylmuramicacid and *N*-acetylglucosamine residues of peptidoglycan, a polymer that is found on the cell wall of bacteria (Buonocore et al., 2014). Transferrin acts through the binding of iron, which creates an environment with lower amounts of free iron, that hinders bacterial growth (Stafford & Belosevic, 2003). The complement system and interferons are also involved in the non-specific immunity (Swain & Nayak, 2009). The complement system is a cascade of proteins that are activated after an encounter with a pathogen, resulting in processes of phagocytosis, inflammation or membrane disruption of the pathogen. Although the complement system belongs to the innate system, it can be triggered through antibodies generated by the adaptative immune system (Boshra et al., 2006).

The adaptative immune system is characterized by a more delayed but specific response, with the action of immunoglobulins, T-cell receptors, cytokines, and major histocompatibility complex molecules. This type of response is dependent on macrophages to present an antigen to T-cells (Swain & Nayak, 2009). Immunoglobulins are proteins that bind to a specific molecule of the pathogen (antigen), and either neutralize them directly or mark them for neutralization by other components of the immune system (Cabillon & Lazado, 2019). Cytokines are small peptides or proteins that play a role on cellular communication, mediating the immune response (Zou & Secombes, 2016). The major histocompatibility complex is involved in the recognition of antigens and their presentation (Dixon et al., 1995).

Nevertheless, the immune system effectiveness relies on the combined action of both innate and adaptative response mechanisms. If a pathogen in fact overcomes the physical barriers, pattern recognition receptors of immune cells detect the agent, which triggers an innate immune response. The subsequent antigen uptake leads to cytokine release, activating cells involved in the inflammatory processes. Furthermore, the presentation of the antigen triggers antigen-specific lymphocytes, with receptors able to identify specific molecules of each pathogen. This leads to secondary responses, implicated in adaptive immunity and responsible for further responses and memory (Cabillon & Lazado, 2019).

In aquaculture, strategies have been studied both for improving the natural capacity of fish to resist to pathogens (immunomodulation) and for counteracting their action when pathogens are indeed able to spread and cause disease. For the prevention and control of most bacterial diseases, antibiotics have been used for several years. However, considering that they promote the development of resistant strains, they are no longer used preventively or as growth promoters in EU (European Union, 2003, 2019). Thus, the intensification of aquaculture systems (in which high densities and frequent handling lead to a negative impact of fish welfare and immune status), has caused a necessity of reliable alternatives that can promote fish robustness minimizing the therapeutic usage of antibiotics (Dawood et al., 2018). For this, the supplementation of aquafeeds with compounds that improve fish resistance to disease (immunomodulators) has been proposed (Raa, 1996). Immunomodulators often act through activation of the immune system as a pathogen would, with an impact on the adaptative immune system and memory cells, rendering a faster and more robust response towards future pathogen contacts (Magnadottir, 2010). Another alternative for immune enhancement is through microbiota modulation, since the commensal microbiota may be an impairment to the growth and spreading of an invasive species (Dawood et al., 2018). Moreover, microbiota affects epithelial renewal and maturation, which in turn has an impact on immune response (Wang et al., 2018). Additionally, considering that the animal stress management in aquaculture is important for its sustainability and economic success, strategies to improve fish welfare and reduce stress have also been proposed. Among these, dietary interventions through the supplementation of nutritional and non-nutritional compounds are reported in the literature (Ciji & Akhtar, 2021).

However, in order to assess whether this approach is valid, the diets must be tested beyond a growth trial, with the fish being subjected to infectious or stress challenges, as the true impact on fish response to this may not be fully perceived without such stimulus. Table 1 summarizes the published reports on dietary intervention for stress mitigation and immunity enhancement in European seabass, published over the last years. These include structural changes in diet formulations, such as changes in the levels of macronutrients or alternative protein or oil sources, or the inclusion of functional additives in the feeds, generally in low (below 5 %) levels. Several stress challenges have been studied, including confinement (increased density), salinity or temperature changes, air exposure, and exposure to different pathogens. These simulate what occurs in fish farms, where manipulation of the animals for zootechnical measurements or transportation is frequent, and also obstacles that can be brought by climate change.

Moreover, the bacteria used in the infectious challenges are species that currently pose a problem in aquaculture, including *Vibrio* bacteria, *Tenacibaculum maritimum* and *Photobacterium damselae* subsp. *piscicida* (*Phdp*). Most of these studies are performed on seabass larvae or juveniles, as these age groups are the ones that present a less developed immune system and higher mortality when exposed to adverse conditions (Gisbert et al., 2014; Rojo-Cebreros et al., 2018).

From the results presented on Table 1, there is a clear need for further dietary manipulations as a way of preventing oxidative stress in European seabass. Most dietary interventions have either a negative or limited impact on oxidative stress. Through the use of animal fats to replace FO, Silva-Brito et al. (2019) did decrease liver lipid peroxidation after a salinity challenge, but plasmatic cortisol was increased. Saleh et al. (2018) found that astaxanthin added to a FM-based diet did increase European seabass survival after an extreme acute salinity change, compared to a non-supplemented control, but no analysis of cortisol or lipid peroxidation were performed. Dinardo et al. (2021) observed that the dietary inclusion of oregano essential oil at 100 ppm led to a decrease in liver LPO after a temperature challenge, mostly due to an increase in the activity of superoxide dismutase (SOD) and catalase (CAT). These are two enzymes that are involved in protection against oxidative stress: SOD converts the highly reactive superoxide radical into molecular oxygen and hydrogen peroxide, and CAT further processes hydrogen peroxide into water and oxygen (Bertocci & Mannino, 2022). In contrast, Islam et al. (2021) and Serradell et al. (2020) found that dietary antioxidants (propolis and vitamins or essential oils, respectively) improved stress response (namely through increased respiratory burst and decreased cortisol, lactate or glucose) without activating the enzymatic antioxidant system, suggesting a sparing effect of dietary antioxidants over enzymatic antioxidants. Nevertheless, no analysis of LPO or other possible damage from ROS was performed. A similar hypothesis is proposed by Serradell et al. (2020), who found that essential oils did not activate, post-stress, the enzymatic antioxidant system, and observed a reduction in post-stress cortisol for fish fed diets with those essential oils. Castro et al. (2018) found that diets supplemented with carbohydrates decreased liver LPO compared to a control diet, after a handling stress, but an increase after the challenge was still found. Zambonino-Infante et al. (2019) also showed that carbohydrates could enhance larvae survival to a hypoxia challenge, but this capacity was lost over time. In contrast, Machado et al. (2019) claims the effect of dietary carbohydrate incorporation on seabass response to a handling stress was not clear, although the same work did suggest that replacing fish oil by vegetable oils affected the immune response in stressed fish. This is in agreement with the results of Pelusio et al. (2022), who demonstrated that replacing FM and FO with vegetable ingredients led to a higher reactivity to acute crowding. Amaral et al. (2023) found that inclusion of fermented plant feed stuff was not effective at mitigating the stress cause by environmental changes. These works highlight the need for better strategies regarding the prevention of stress damage in fish when working with high inclusion levels of plant-based ingredients.

According to literature, several ingredients have demonstrated potential to reduce mortality in experimental infectious challenges (table 1): galactomannan–oligosaccharides (Serradell et al., 2022; Serradell et al., 2020; Torrecillas et al., 2019), plant essential oils or extracts (Bilen et al., 2019; Serradell et al., 2022; Serradell et al., 2020; Torrecillas et al., 2019), shrimp hydrolysates (Gisbert et al., 2018), garlic powder combined with chitosan (Abdel-Tawwab et al., 2020), algae blends (Ferreira et al., 2023; Güroy et al., 2022), pre- and probiotics (Monzón-Atienza et al., 2022; Torrecillas et al., 2018) and methionine (Machado et al., 2018).The improvements in resistance towards infection can be a result of non-specific immune markers enhancement (Bilen et al., 2019; Gisbert et al., 2018; Machado et al., 2018; Monzón-Atienza et al., 2022), stimulation of cells of the immune systems (Abdel-Tawwab et al., 2020; Serradell et al., 2020), modifications in the inflammatory response towards the pathogen (Ferreira et al., 2023; Güroy et al., 2022; Machado et al., 2018; Serradell et al., 2022; Torrecillas et al., 2018) or alterations in the gut health (Torrecillas et al., 2019). In contrast, Rivero-Ramírez et al. (2020) did not find increased resistance to *V. anguillarum* infection by dietary inclusion of arachidonic acid. These studies focus mostly on the inclusion of a functional ingredient to the basal diet, rather than a significant change in the main protein or lipid source.

Table 1 - Studies assessing the impact of dietary modifications in European seabass response to an environmental stressor or infectious challenge.

Fish size (g)	Tested ingredient	Inclusion levels	Stressor	Impact on growth performance	Impact on immune markers	Impact on oxidative stress	Ref.
Larvae (from mouth opening)	Carbohydrates	34%	Hypoxia for 1 h (6 and 9 months after returning to basal diet)	↑ amylase and sucrase activity in pancreatic and intestinal segments, during the first period of feeding ↓ growth due to ingredient, during the first period of feeding ↓ survival due to ingredient, during the first period of feeding = final weight during the second period of feeding	n.a.	↑ tolerance to hypoxia, due to ingredient, in the first challenge = tolerance to hypoxia, in the second challenge ↑ plasma glucose due to ingredient, during the second period of feeding ↑ liver glycogen due to ingredient, during the second period of feeding	(Zambonino-Infante et al., 2019)
9.8	FM and FO	20% or 5% and 6% or 3%, respectively	Infectious (*V. anguillarum*) for 7 days	↑ final weight due to FM = final weight due to FO	↓ *il1β, tnfα, mhcII* and *cox2* due to FM ↑ post-infectious survival due to FO and FM ↓ *ex vivo* and *in vivo* gut bacterial translocation rates	n.a.	(Torrecillas et al., 2017a)
20.9	Fermented plant feedstuff	20%	Salinity (fixed vs oscillatory); temperature (21 vs 26 °C) for 6 weeks	= body weight ↓ growth rate due to ingredient and salinity ↓ feed efficiency due to salinity ↓ feed intake due to ingredient	↓ peroxidase due to temperature ↓ lysozyme due to temperature ↑ lysozyme due to salinity ↓ ACH50 due to salinity ↑ ACH50 due to ingredient	Interaction for temperature and diet for GPx and CAT	(Amaral et al., 2023)
62.6	Animal fats	75% or 100% FO replacement	Salinity change (15‰) for 72 h	= final weight and HSI	n.a.	↓ plasma osmolality due to challenge ↓ plasma osmolality due to ingredient, 24h post-challenge ↑ plasma cortisol over time, in 100% animal fat ↑ in plasma lactate due to salinity ↑ plasma lactate for the 75% animal fat diet, 72h post-challenge	(Silva-Brito et al., 2019)

Fish size (g)	Tested ingredient	Inclusion levels	Stressor	Impact on growth performance	Impact on immune markers	Impact on oxidative stress	Ref.
						= plasma glucose due to ingredients ↓ Liver LPO due to ingredient ↓ glucose due to challenge ↑ GR activity in control diet ↑ post-challenge citrate synthetase activity in 100% animal fat diet = amylase and lipase due to challenge and ingredient ↑ proteases activity due to salinity	
74.0	Vegetable oils and carbohydrates	10% and 20%, respectively	1-min air exposure followed by tank change for 1 h	= growth performance	↓ haematocrit and peroxidase due to oils ↓ head kidney *mc2r* and *gr1* due to oils ↓ neutrophils and plasma antiproteases activity due to oils ↓ post-stress head kidney *cox2* due to oils ↓ post-stress head kidney *mc2r* due to carbohydrates ↓ plasma bactericidal activity due to ingredients interaction ↑ plasma peroxidase due to ingredients interaction ↑ post-stress plasma antiprotease activity due to carbohydrates	↑ cortisol due to stress = cortisol due to diets ↓ post-stress plasma NO due to carbohydrates	(Machado et al., 2019)
72.3	FM and FO	30/15, 20/7 or 10/3 (%/%)	Confinement (final density 80 kg m^{-3}) for 2 h	↓ final weight, weight gain and SGR in the 10/3 group	↑ skin mucosal IgM and bactericidal activity in the 10/3 group	↑ post-stress lactate in the 30/15 group ↑ post-stress *hps70* in the 30/15 group ↑ *hsp70*, *gr1* and *gr2* due to stress ↓ triglycerides, alanine	(Pelusio et al., 2022)

Fish size (g)	Tested ingredient	Inclusion levels	Stressor	Impact on growth performance	Impact on immune markers	Impact on oxidative stress	Ref.
						transaminase, creatine kinase, lactate dehydrogenase, phosphorus and potassium in plasma due to stress ↑ protein, sodium and cortisol in plasma due to stress	
0.4	astaxanthin	60, 80, 100 mg/kg	Salinity change (0.3‰) for 24 h	↑ final weight and SGR due to ingredient ↓ FCR and feed intake due to ingredient ↑ body protein due to ingredient	↑ lysozyme due to ingredient	↓ basal SOD and GPx due to ingredient ↑ post-challenge survival due to ingredient	(Saleh et al., 2018)
0.75	n-3 PUFA	0.73 or 1.65%	Temperature (15 vs 20 °C), for 150 days	↑ Liver mass at 20 °C = liver mass due to ingredient	n.a.	↑ peroxidation index for the 1.65% diet = MDA and citrate synthetase at all temperatures and diets ↑ cytochrome c oxidase at 20 °C ↑ CAT in the 1.65% diet ↓ SOD in the 1.65% diet ↑ SOD at 20 °C	(Gourtay et al., 2020)
5.7	Spirulina	1, 2.5 and 5%	Infectious (*Vibrio anguillarum*) for 7 days	= final weight and SGR ↓ FCR for the 1% diet ↑PER for the 1% diet	↑ lysozyme for the 5% diet ↑ myeloperoxidase for the 2.5 and 5% diets ↑ *il1β* for the 1% diet in spleen = *il1β* in the intestine ↑ *il6* and *il8* in head kidney and intestine due to ingredient ↑ *il10* in head kidney for the 1% diet ↑ *il10* in the intestine due to ingredient = *tnfα* in all tissues ↑ tgfβ in kidney and intestine	n.a.	(Güroy et al., 2022)

Fish size (g)	Tested ingredient	Inclusion levels	Stressor	Impact on growth performance	Impact on immune markers	Impact on oxidative stress	Ref.
					due to ingredient = hepcidin in all tissues ↑ post-infectious survival due to ingredient		
8.4	Arginine and citrulline	0.5 or 1%	Infectious (*Photobacterium damselae piscicida*) for 48 h	= growth performance due to ingredient	= haematological parameters due to ingredient ↓ post-infectious neutrophils and monocytes in 1% arginine diets = immune and inflammatory markers	n.a.	(Azeredo et al., 2020)
8.5	Methionine	0.5 or 1%	Infectious (*Photobacterium damselae* subsp. piscicida) for 21 days	= final weight	↑ post-infection survival due to methionine ↑ basal white blood cells due to methionine ↑ basal neutrophils in the 1% diet = basal ACH50 due to diets ↓ basal lysozyme due to diets ↑ basal bactericidal activity in the 1% diet ↓ head kidney *il1β, noxin, casp-3* and *sat1* due to methionine ↑ post-infectious white blood cells due to methionine ↑ post-infectious neutrophils due to methionine ↑ post-infectious lysozyme due to methionine ↑ post-infectious ACH50 for 0.5% diet ↓ post-infectious head kidney *casp-3* and *mTOR*	n.a.	(Machado et al., 2018)
8.9	Vitamin C and E; propolis; Phycocyanin; β-	0.3-0.45%	Temperature (22 to 32 °C, increasing at 3.5	↑ body weight due to propolis, vitamins and phycocyanin = feed intake	↑ post-stress red blood cells, hematocrit, hemoglobin, serum lysozyme	↑ respiratory burst due to propolis, vitamins and phycocyanin	(Islam et al., 2021)

Fish size (g)	Tested ingredient	Inclusion levels	Stressor	Impact on growth performance	Impact on immune markers	Impact on oxidative stress	Ref.
	glucan		°C day^{-1}) for 18 days		activities and *igf1* due to propolis, vitamins and phycocyanin	↓ glucose and lactate due to propolis, vitamins and phycocyanin ↓ *hsp70*, SOD, GPx, CAT and GR due to propolis and vitamins	
11.3	Algae blend	2%, 4% or 6%	Infectious (*Tenacibaculum maritimum*) for 8 days	↑ final weight and SGR due to ingredient	↑ post-infectious *mmp9* and *pcna* due to ingredient ↑ post-infectious *il1β* and *il8* due to ingredient ↑ post-infectious plasma bactericidal activity due to ingredient ↑ post-infectious head kidney hepcidin due to ingredient ↑ peripheral neutrophils for the 4% diet ↑ acid goblet cells in the intestine for the 4% diet ↑ post-infectious survival for the 4% diet	n.a.	(Ferreira et al., 2023)
12.0	Tryptophan	0.3%	Confinement for 15 days (final density 10 kg m^{-3}) followed by infection (*Phdp*)	n.a.	↑ post-infectious *il1β* and *tgfβ* (at 4 h), *tnfα* (at 24 h) and *cd8α* and *-β* (at 48 h) expression due to stress ↓ plasma bactericidal activity due to tryptophan ↓ post-infectious *il1β, cd8α* due to tryptophan ↑ Post-infectious *tgfβ, cxcr4, cd8α, cd8β, cd3ζ, tcrα, afmid, ido2* due to tryptophan in confined fish ↓ post-infectious *tgfβ, tnfα, cd8β* due to tryptophan in confined fish ↓ post-infectious MCV and SOD due to tryptophan	↓ activity of SOD, CAT and TG due to stress ↓ post-infectious cortisol due to tryptophan in confined fish	(Machado et al., 2022)

Fish size (g)	Tested ingredient	Inclusion levels	Stressor	Impact on growth performance	Impact on immune markers	Impact on oxidative stress	Ref.
					↓ haemoglobin, haemoglobin corpuscular indexes due to stress		
12.5	Oregano essential oil	100 or 200 ppm	Temperature (13 to 25 °C) for 150 days	↑ body weight and SGR for the 100 ppm diet	n.a.	↓ serum TBARS in the 100 ppm diet ↑ serum TBARS due to temperature ↑ SOD and CAT due to temperature until 18 °C ↓ SOD and CAT due to temperature from 18 to 25 °C ↑ SOD and CAT in the 100 ppm diet ↑ Plasma protein due to ingredient ↓ triglycerides due to ingredient and temperature ↓ cholesterol in the 100 ppm diet	(Dinardo et al., 2021)
13.4	arachidonic acid	0.5, 1, 2, 4 or 6%	Infectious (*Vibrio anguillarum*) for 1 week	↓ final weight for the 0.5% diet	= survival to challenge ↑ basal *il1β* in 0.5% diet ↑ post-infectious *il1β* in 0.5% and 2% diet ↓ post-infectious *il10* for the 2% diet ↑ post-infectious *cox2* for the 2% diet	n.a.	(Rivero-Ramírez et al., 2020)
19.7	Prebiotic (Biomos® and Actigen ©) and/or probiotic (Bactocell ®)	0.6 or 0.3% for prebiotic, undescribed for probiotic	Infectious (*V. anguillarum*) for 6 days	↑ final weight due to prebiotic, regardless of level = somatic indexes and SGR = posterior gut morphometry and mucus production	↑ *tnfα, cox2, cd4, il10* due to prebiotic (0.6%) ↑ *il1β* and *cox2* due to probiotic ↑ post-infection survival due to ingredients	n.a.	(Torrecillas et al., 2018)
19.8	Shrimp protein hydrolysate	5%	Confinement for 10 days (final density 29.6 kg m^{-3}) followed by	= final weight and SGR ↓ FCR due to ingredient = proximate composition	↑ lysozyme, ACH50 and bacteriolytic activity in serum due to ingredient ↑ survival to infection due to	n.a.	(Gisbert et al., 2018)

Fish size (g)	Tested ingredient	Inclusion levels	Stressor	Impact on growth performance	Impact on immune markers	Impact on oxidative stress	Ref.
			natural outbreak of *Vibrio pelagius*		ingredient		
19.9	*Cotinus coggygria* or *Malva sylvestris* extracts	500 or 1000 mg/kg	Infectious (*Vibrio anguillarum*) for 2 weeks	↑ final weight and feed intake due to *Malva sylvestris* = FCR for all diets	↑ Oxidative radical production and phagocytic activity in leucocytes due to ingredient, more for *Cotinus coggygria* 500 mg/kg ↑ lysozyme and myeloperoxidase activity due to ingredients ↑ bactericidal activity of macrophages due to ingredients, more for 1000 mg/kg ↑ post-infectious survival due to ingredients	n.a.	(Bilen et al., 2019)
23.5	Galactomannan oligosaccharides or mix of garlic and labiate essential oils	5000 ppm and 200 ppm, respectively	Infectious (*V. anguillarum*) combined with confinement (final density 35 kg m^{-3}) for 7 days	= final weight, SGR, FCR = mucosal fold length ↓ fold area covered by mucus, due to ingredients ↓ goblet cell size in posterior intestine due to ingredients ↓ rectum submucosa due to oligosaccharides = granulocytes and lymphocytes infiltration in intestine ↑ microvilli length due to oligosaccharides	↑ post infectious survival due to ingredients = share of posterior gut fold area covered by goblet cells due to challenge ↑ share of mucus coverage in the posterior intestine 2h and 7 days post-infection, due to essential oils ↓ share of mucus coverage in the posterior intestine 24h post-infection, due to essential oils ↑ goblet cell size 24h after infection, due to essential oils	N.a.	(Torrecillas et al., 2019)
23.5	Galactomannan oligosaccharides or mix of garlic and labiate essential	5000 or 200 ppm, respectively	Confinement for 1 week (final density 34.6 kg m^{-3}) combined with infection	= growth performance	↑ post-infectious survival for both ingredients ↓ post stress *cyp11b*, *hif-1α*, *casp-3* and *il1β*, in confined-only fish fed essential oils	= basal cortisol ↑ cortisol with stress ↓ post-stress cortisol for both diets in confined-only fish	(Serradell et al., 2020)

Fish size (g)	Tested ingredient	Inclusion levels	Stressor	Impact on growth performance	Impact on immune markers	Impact on oxidative stress	Ref.
	oils		(*Vibrio anguillarum*) or alone		↑ *StAR* in confined-only fish fed essential oils. ↓ *StAR* and *casp-3* in infected fish fed essential oils ↑ post-infectious lysozyme for both diets	= glucose due to stress or diets in confined-only fish ↓ glucose 2h post-challenge in infected fish due to ingredients ↑ glucose 1 week post-challenge in infected fish due to ingredients	
23.5	plant-derived galactomannan – oligosaccharides;mix of garlic and labiate plant essential oils	0.05% 0.02%	Confinement for 7 days (final density 35 kg m^{-3}) combined or not with infectious challenge (*V. anguillarum*)	= growth performance	↑ survival in the infectious challenge due to ingredients Attenuated pro-inflammatory response due to ingredient	= basal plasmatic stress parameters ↓ post-stress cortisol and glucose due to ingredients Attenuated stress response due to oligossacharides No activation of endogenous antiox system due to essential oils	(Serradell et al., 2022)
26	*B. velezensis* (probiotic)	10^6 CFU g^{-1}	Infectious (*V. anguillarum*-LPS and live challenge) for 6 days	n.a.	↑ plasma bactericidal activity and lysozyme ↑ *il1β*, *tnfα*, *cox2* and dicentracin in LPS-treated fish ↑ survival to *V anguillarum*	n.a.	(Monzón-Atienza et al., 2022)
30.7	Garlic powder or chitosan	30 or 10 g/kg, respectively	Chemical (zearalenone) for 4 weeks and infectious (*V. alginolyticus*) for 2 weeks	↓ final weight and SGR due to chemical stress ↑ final weight and SGR due to ingredients, more if combined ↓ feed intake due to chemical stress ↑ feed intake due to ingredients, more if combined	↓ red blood cells, white blood cells, haemoglobin, haematocrit, mean corpuscular haemoglobin concentration due to chemical stress ↑ MCV and mean corpuscular haemoglobin due to chemical stress ↓ total protein, albumin, globulin and lysozyme due to chemical stress ↑ red blood cells, haemoglobin, mean corpuscular haemoglobin	n.a.	(Abdel-Tawwab et al., 2020)

Fish size (g)	Tested ingredient	Inclusion levels	Stressor	Impact on growth performance	Impact on immune markers	Impact on oxidative stress	Ref.
					concentration and white blood cells due to ingredients ↓ post-infectious survival due to chemical stress ↑ post-infectious survival due to ingredients, more if combined		
73.0	Salmon hydrolysates	5 or 10%	Temperature (30.5 °C) and hypoxia (64.6%) for 7 days	= SGR, final weight and somatic indices = FCR, intake, PER. ↑ LER for the 10% diet	↓ lactate due to hydrolysate ↑ *il1β* and *il8* in 10% diet ↑ *il10* in 5% diet ↓ ferritin, *mx* and *il8* due to stress	↑ plasma cholesterol and potassium in 5% diet ↓ post-stress (temperature and hypoxia) triglycerides in the 5% diet ↓ plasma glucose in 10% diet ↓ plasma urea and triglycerides due to stress ↑ alkaline phosphatase due to stress = cortisol	(Parma et al., 2023)
92.6	Taurine	1.5%	Forced swimming for 3 h	↑ final weight due to ingredient = FCR	n.a.	=metabolic oxygen consumption during exercise ↑ critical swimming speed due to ingredient ↓ respiratory burst activity due to ingredient ↓ liver *cat* and muscle *gpx* due to ingredient = liver *gpx, sod* = muscle *cat* and sod	(Ceccotti et al., 2019)
274.7	Methionine and tryptophan	1.16 and 0.52%, respectively	Infectious (*Photobacterium damselae* subsp. *piscicida*) for 24 h	n.a.	↑ complement factor 3 due to methionine ↑ post-infectious neutrophils, monocytes and lymphocytes due to methionine	= basal neuroendocrine intermediates ↑ post-infectious cortisol due to ingredients ↑ brain monoamine content due to tryptophan = total antioxidant capacity in liver	(Azeredo et al., 2017a)

Fish size (g)	Tested ingredient	Inclusion levels	Stressor	Impact on growth performance	Impact on immune markers	Impact on oxidative stress	Ref.
						↑ peripheral blood respiratory burst in tryptophan, compared to methionine ↓ post-infection *gr* due to ingredients	

ACH50 – alternative complement pathway; afmid – Arylformamidase-like; casp-3 – caspase-3; CAT – catalase; cd3ζ – cluster of differentiation 3 ζ chain; cd4 – Cluster of differentiation 4; cd8α – cluster of differentiation 8 α chain; cd8β – cluster of differentiation 8 β chain; cox2 – Cyclooxigenase-2; cxcr4 – Chemokine cxc receptor 4; cyp11b – cytochrome P450 family 11 subfamily b; FCR – feed conversion ratio; FM – fishmeal; FO – fish oil; G6PD – Glucose-6-phosphate dehydrogenase; GPx – glutathione peroxidase; gr-1 – glucocorticoid receptor 1; gr-2 – glucocorticoid receptor 2; GR – glutathione reductase; h1f-1α – hypoxia inducible factor; HSI – hepatosomatic index; hsp70 – heat shock protein 70; ido2 – indoleamine dioxygenase 2; igf1 – insulin-like growth factor 1; IgM – Immunoglobulin M; il1β – interleukin 1 β; il6 – interleukin 6; il8 – interleukin 8; il10 – interleukin 10; LER – lipid efficiency ratio; LPO – lipid peroxidation; LPS – lipopolysaccharide; mc2r – Melanocortin 2 receptor; MCV – mean corpuscular volume; MDA – Malondialdehyde; mhcII – Major histocompatibility complex II; mmp9 – Matrix metalloproteinase; mTor - Mechanistic target of rampamycin; mx – mx protein; NO – nitric oxide; noxin – nitric oxide inducible gene protein; pcna – Proliferating cell nuclear antigen; PER – protein efficiency ratio; PUFA – polyunsaturated fatty acids; sat1 – spermine/spermidine N (1)-acetyltransferase; SGR – specific growth rate; SOD – superoxide dismutase; STaR – steroidogenic acute regulatory protein; TBARS - thiobarbituric acid reactive substances; tcrα – T-cell receptor α chain; TG – total glutathione; tgfβ – Transforming growth factor β; tnfα – Tumor necrosis factor α. N.a. – Not available.

As the trend towards increasing the level of plant ingredients in aquafeeds continues, there is a growing demand for novel functional additives. Indeed, the global feed additives market has an estimated compound annual growth rate (CAGR) of 5.8% from 2023 to 2028 (Figure 2). This market is estimated to reach 55.8 USD billion by 2028 (Markets and Markets, 2022).

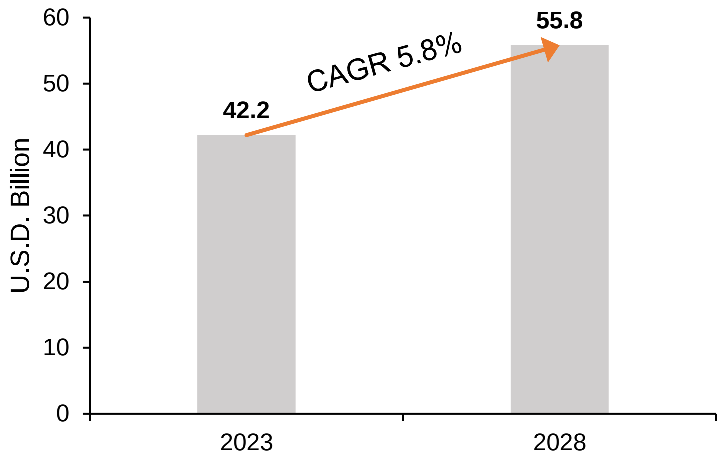

Figure 2 – Global market for feed additives, which has a compound annual growth rate (CAGR) of 5.8%. Adapted from Markets and Markets (2022).

In accordance with the EU regulation (European Union, 2003), feed additives can be classified based on their function into technological additives, sensory additives, zootechnical additives, nutritional additives and coccidiostats and histomonostats. Its bioactivity is obviously dependent on the chemical composition and structure, but they may be an important ally to supplement plant-based diets while minimizing the negative impacts of these aquafeeds.

1.3.1. Definition, sources and production methods of bioactive peptides

Bioactive peptides (BP) are molecules, derived from proteins, composed by a small AA chain (2-20 monomers), which have a potential benefit for an organism's health or well-being (Chalamaiah et al., 2019; Liu et al., 2020), beyond the nutritional AA content. Their application in functional foods, nutraceuticals, cosmetics and therapeutics has been widely studied (Aguilar-Toalá et al., 2019; Baig et al., 2018; Li-Chan, 2015). The interest in these molecules has been on the rise due to their wide range of bioactivities (Table 2).

Table 2 – Examples of potential properties of bioactive peptides.

Property	Animal model	Ref.
✓ Anti-aging	Fruit flies and mice	Lin et al. (2020); Yazaki et al. (2017)
✓ Anti-diabetic (hypoglycaemic or anti-hyperglycaemic)	Mice	Casanova-Martí et al. (2019); Harnedy et al. (2018)
✓ Antihypertensive (angiotensin-I-converting enzyme inhibitors)	Rats	Guo et al. (2020); Suárez et al. (2020)
✓ Anti-inflammatory	Mice; piglets	Chung et al. (2020); Fu et al. (2023)
✓ Antimicrobial	In vitro; chicken	Shen et al. (2022); Tai et al. (2020)
✓ Anti-obesity	Mice, rats and macaques	Zhanzhan et al. (2023); Zhao et al. (2019)
✓ Antioxidant	Zebrafish; broiler chickens	Aslam et al. (2020); Ding et al. (2019)
✓ Cancer preventive or delayer	Mice	Koide et al. (2023); Li et al. (2019)
✓ Hypocholesteraemia	Rats	Allaoui et al. (2018); Aloysius et al. (2022)
✓ Immunomodulatory	Mice	Khan et al. (2022); Lin et al. (2023)
✓ Mineral-binding	Zebrafish; rats	Gan et al. (2023); Sun et al. (2021)

BP can be obtained from an enormous variety of resources through hydrolysis or fermentation processes. Protein hydrolysates are defined as "products obtained by either chemical or enzymatic cleavage of peptide bonds of protein that are composed of free amino acids, peptides and residual intact protein in different proportions" (EFSA Panel on Dietetic Products Nutrition and Allergies, 2021). BP can also be chemically synthesized or extracted from natural sources (Figure 2). In particular, since these peptides are usually encoded in the sequence of proteins, wasteful protein-rich matrices have the potential to be reutilized as raw materials for the production of valuable BP through hydrolysis, which is in line with circular economy policies. This reuse creates added value for high-protein by-products, while allowing the reduction of organic waste (Faustino et al., 2019). Examples of such by-products include fishbones, animal blood, feathers, seeds, okara or fruit peels (Callegaro et al., 2019; Fu et al., 2019; Hernández-Corroto et al., 2020; Urbizo-Reyes et al., 2019; Voss et al., 2019; Zhang et al., 2019).

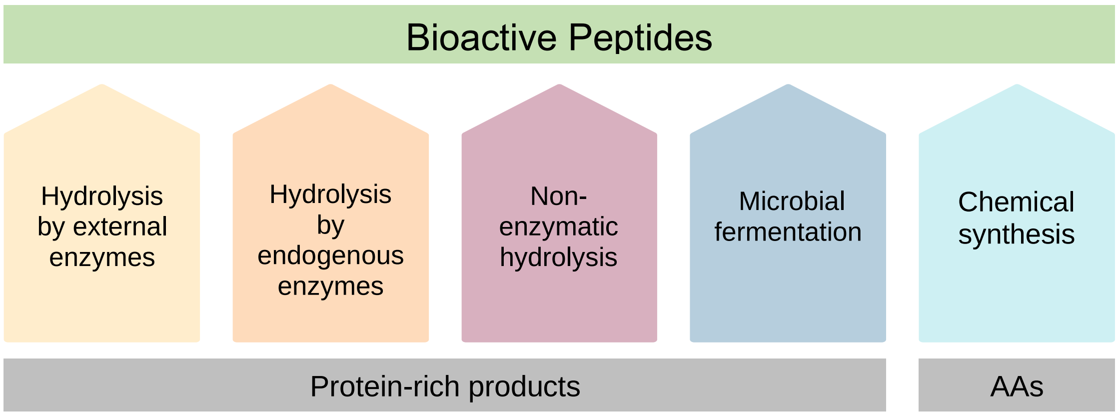

Figure 3 - Processes to obtain bioactive peptides.

Hydrolysis by external enzymes

One of the most common ways to obtain protein hydrolysates is through a reaction with the aid of enzymes, added to the substrate mixture, under a controlled pH and temperature, to maximize their activity (Anderssen & McCarney, 2020). Proteases are a group of hydrolases that catalyse the breakdown of proteins into either AA or small peptides and are extensively used in the industry, such as in detergents, leather treatments or food processing (Tacias-Pascacio et al., 2020). They can be classified into endoproteinases, if they cleave at specific residues within the protein molecule, producing large peptides, or into exopeptidases, if they remove AA from either the C or N terminus (Tacias-Pascacio et al., 2020). Specificity of proteases is highly variable, with some hydrolysing bonds adjacent to a precise AA residue contrarily to others. However, this is not the only factor with an impact in the resulting peptide profile; environmental features also play an important role, particularly pH and temperature, which greatly affect the reaction kinetics (Wubshet et al., 2019). In the production of BP, the use of either a single protease or a combination of proteases (simultaneously or sequentially) is possible and can yield different peptides at the end of the process (Hou et al., 2023; Kurniadi et al., 2023). The use of an extract, rich in proteases, has also been proposed (Voss et al., 2019).

Hydrolysis by endogenous enzymes

An alternative to the application of external enzymes to catalyse the hydrolysis process is to rely on enzymes naturally present on the substrate. This can be achieved by subjecting the protein substrates to specific environmental conditions of temperature, pH or solid contents, in order to maximize the activity of such enzymes (Toldrá et al., 2020). This type of hydrolysis can be combined with the addition of external enzymes, either simultaneously, or subsequently (Mirzaei et al., 2015; Samaranayaka & Li-Chan, 2008). BP from shrimp heads, fish (*Leiognathus splendens*) and spent brewer's yeast have been produced through this process (Amorim et al., 2019; da Silva et al., 2017; Prabha et al., 2020).

Non-enzymatic hydrolysis

The release of BP from proteins can be achieved without resourcing to enzymatic treatment. Acid hydrolysis is a way of obtaining BP by addition of acids such as hydrochloric or sulfuric acid (Hou et al., 2017). Acid hydrolysates are commonly applied in food and pet food industries as flavour enhancers (Hou et al., 2017; Nagodawithana et al., 2008). Alternatively, alkaline hydrolysis can be achieved by addition of an alkaline substance (Connolly et al., 2013; Ulug et al., 2021). Alkaline hydrolysis can, however, generate undesired compounds such as lysioalanine, ornithinoalanine, lanthionine and ß-aminoalanine. Moreover both acid and alkaline hydrolysis display several disadvantages: they can result in loss of some AA, like tryptophan; they can be considered ecologically questionable; they can yield higher amounts of free AA rather than peptides; the final product characteristics are harder to control; and the final products have high salt amounts, due to neutralization steps and the end (Hou et al., 2017; Ulug et al., 2021). In any case, chemical processes for obtaining peptides are often preferred by the feed additives industry, as they are operationally simple and have a low cost (Anal et al., 2013; Ulug et al., 2021).

Subcritical water extraction, also known as autohydrolysis, is a process that relies on the use of water below its subcritical point (22.1 MPa and 374 °C), being considered an environmentally friendly extraction process due to the avoidance of organic solvents (Ahmed & Chun, 2018; Nastić et al., 2018). It has been hypothesized that this process could be used for the extraction of AA or BP from protein-rich matrices, either by itself or combined with an enzymatic method (Fan et al., 2020; Marcet et al., 2014; Rivas-Vela et al., 2023; Wael & Hiroyuki, 2013), although this process is still not commonly used by the hydrolysate industry.

Microbial fermentation

Fermentation refers to the incubation of the matrices to be hydrolysed with specific microorganisms under defined conditions (Gao et al., 2021), taking advantage of the microbial proteases these microorganisms possess. *Lactobacillales* are an order of bacteria that can be used for this process (Akbarian et al., 2022). They are well established within the food and feed industry and have a generally recognized as safe (GRAS) status (Ringø et al., 2020). BP from milk, soybean meal, whey, or amaranth have been obtained from fermentation (Cruz-Casas et al., 2023; Helal et al., 2023; Kumari et al., 2023; Manzanarez-Quin et al., 2023). In the particular case of aquafeeds, fermented soybean meal rich in bioactive peptides has been studied in diets for Japanese flounder, *Paralichthys olivaceus* (Abdul Kader et al., 2012). A fermented pellet feed rich in BP has also been investigated for common carp *(Cyprinus carpio)* (Zhang et al., 2023). Fonseca et al. (2023) also has suggested the application of fermented algae as an ingredient for European seabass diets.

Post-processing

When hydrolysing protein rich matrices using the aforementioned methods, the final hydrolysate contains several peptides, which can possess different bioactivities. This hydrolysate can be purified or concentrated, to several degrees, as it often contains other co-extracted compounds (Echave et al., 2021). Membrane filtrations are generally applied for this matter. The basis is the selective permeability of some of the components of a liquid mixture through a membrane, depending on their size, charge, shape or other properties (Alavi & Ciftci, 2023). Considering the membrane pore size, these filtrations can be classified as micro-(0.1-10 μm), ultra- (1-100 nm) or nanofiltration (1-10 nM) (Alavi & Ciftci, 2023). Often, for food and feed purposes, a hydrolysate is not purified up to individual peptides, as the process would be very expensive. On the other hand, combining different compounds can often result in interesting synergetic activities.

1.3.2. Bioactive hydrolysates in aquafeeds

Various hydrolysates have been produced using the aforementioned methods and studied as potential ingredients in aquafeeds. Table 3 summarises the recent applications of hydrolysates in feeds for European seabass reported in the literature. While sustainable hydrolysates have the potential to replace FM (Cao et al., 2020), this approach is frequently economically impracticable, particularly when an enzymatic process is used. Therefore, that utilization of hydrolysates is mostly reserved to larvae diets, as evidenced in table 3. Larvae production involves minimal amounts of feed, compared to on-growing stages, resulting in a cost-effective approach. Moreover, larvae generally display high mortality and poor resistance towards external stressors and infections. In European seabass larvae, a low inclusion level of hydrolysates seems to improve the growth, diminish the rate of malformations and help with the development of gut maturation (Cahu & Infante, 1995a; Cahu et al., 1998; Delcroix et al., 2015; Skalli et al., 2014). Nonetheless, high levels of inclusion, particularly when replacing fishmeal, may have negative impacts, which once again shows the adequateness of fishmeal for seabass feeding, even from the early stadiums (Cahu et al., 2004; Cahu & Infante, 1995b; Cahu et al., 1999; Infante et al., 1997; Kotzamanis et al., 2007). Delcroix et al. (2015) tested several marine hydrolysates in compound diets for larvae and concluded that the impacts on growth are highly variable on hydrolysate properties and origins – the size fractionation is not the only parameter to take into account, since having smaller peptides did not necessarily imply better results in fish performance. When using hydrolysate as FM replacement, the AA composition of the BP is also essential, to avoid poor protein deposition ratio, and to ensure the species AA requirements are met, as seen in the work of Langar et al. (1993). Additionally, considering that larvae are highly susceptible to infections, hydrolysates with BP may also play a role as immunomodulators, increasing resistance to infections, as observed in Kotzamanis et al. (2007).

Protein hydrolysates may complement alternative protein sources in order to minimize the negative effects that FM replacement might cause. Given the richness of hydrolysates in BP, they emerge as interesting functional ingredients that could potentially confer benefits to fish. These advantages may include increased resistance to diseases or oxidative stress, enhanced digestibility and mineral uptake, and augmented growth performance. Thus, incorporating hydrolysates with bioactive properties in plant protein-rich diets holds great potential for mitigating the consequences of FM replacement, especially at low inclusion levels (below 5%). This approach may become economically viable, as the required amount of the hydrolysate to achieve the desired bioactive effect could be minimal. In addition, if confirmed functional properties lead to diminished mortality or other benefits, they could also contribute to additional economic benefits. Studies involving hydrolysates as functional ingredients often focus on juveniles, assessing their impact on growth, nutrient digestibility and utilisation, stress response, immunity and metabolism (table 3). Indeed, hydrolysates, probably due to the immunomodulatory properties of their BP, have shown promising outcomes in improving fish resistance to pathogens, such as *Vibrio* bacteria (Gisbert et al., 2018; Kotzamanis et al., 2007). Leduc et al. (2018b) showed that a shrimp hydrolysate could up-regulate several genes involved in immune response. Furthermore, Costa et al. (2020) reported that supplementing plant protein-based diets with marine hydrolysates resulted in increased levels of plasmatic lysozyme compared to non-supplemented diets, and similar to those observed in a high FM diet. Lysozyme serves as a marker for the non-specific immunity of fish, suggesting these hydrolysates may give fish the capacity to resist infection similarly to a FM-based diet, even at a low inclusion level of 3%.

Table 3 - Application of protein hydrolysates in feeds for European seabass.

Average fish weight (initial; final), g	Hydrolysate	Inclusion levels	Effects on growth	Effects on nutrient utilisation	Effects on physiology aspects	Ref.
Larvae						
Unknown; 81 mg	Commercial enzymatic fish hydrolysate; three mollusc hydrolysates (H1, H2, H5); one fish hydrolysate (H3); one shrimp hydrolysate (H4)	100-150 g kg^{-1} (to balance nitrogen level) replacing the commercial hydrolysate	↓ survival rate for H1 diet = final weight on larvae fed H3 and the commercial hydrolysate ↑ final weight for H4 and H5 ↓ final weight for H1 and H2	n.a.	↓ trypsin secretion for H2 diet ↓ amylase secretion for H1 diet More advanced gut maturation for H3 and H4 diets ↑ genes involved in inflammation and antioxidative responses for H1 ↓ osteocalcin for H1 ↑ activity of brush border enzymes for H3 and H4 diets The gut bacterial community profiles in H3 and H4 were the only not dissimilar ones H1, H2 and H5 had the most dissimilar microbiota	(Delcroix et al., 2015)
0.49 mg; 9.2 mg	Commercial enzymatic fish hydrolysate; yeast hydrolysate; pig blood hydrolysate; pig red blood cells hydrolysate	12% for the commercial hydrolysate; 6 or 12% for all other hydrolysates; replacing FM	= final weight between commercial and 6% yeast, 6% pig blood and both red blood cell hydrolysates ↓ growth for 12% pig blood and yeast hydrolysate = survival for 6% of all hydrolysates, ↓ survival for the 12% of all hydrolysates ↑ incidence of malformations for the 12% pig blood hydrolysate ↓ incidence of malformations for the 6% yeast hydrolysate	n.a.	= Trypsin activity and secretion = AP/leu-ala ratio (and intestinal maturation) = Amylase secretion ↑ amylase activity for the 12% yeast hydrolysate	(Skalli et al., 2014)
<1 mg; 18 mg	Commercial fish hydrolysate (longer peptides) and an experimental freeze-dried hydrolysate from acidic silage of	10 or 19%, replacing FM	= Survival rate ↑ Growth for the 10% commercial hydrolysate ↓ growth for the 10% experimental hydrolysate	N.a.	= Trypsin and amylase secretion ↑ intestinal enzyme (AP and lap) activity for the 10% commercial hydrolysate Predominance of *Vibrio* spp. in the microbiota for the experimental	(Kotzamanis et al., 2007)

Average fish weight (initial; final), g	Hydrolysate	Inclusion levels	Effects on growth	Effects on nutrient utilisation	Effects on physiology aspects	Ref.
	sardine offal (smaller peptides)				hydrolysates Predominant bacteria in 10% commercial hydrolysate was *Marinomonas* spp *Bacillus* spp. were dominant in the 19% commercial hydrolysate Lower mortality rate for the 19% sardine hydrolysate, after 3 days of challenging with *V. anguillarum*. Hydrolysate dose had no effect on mortality after infection.	
1 mg; 14 mg	Hydrolysed FM	14%; 46%; 10% + potato starch; replacing FM	↑ growth for the 14% diet ↓ survival rate for the 46% diet	n.a.	↓ pancreatic trypsin activity for the 46% diet ↓ cholecystokinin for the 46% diet ↓ trypsin and amylase for the 46% diet	(Cahu et al., 2004)
0.5 mg; 4.5 mg	Commercial enzymatic fish hydrolysate	19, 38.5 or 58%, replacing FM	= growth for the 19% diet and diet without hydrolysate ↓ growth for 38.5% and 58% diets ↓ survival for the 58% diet ↑ survival for the 19% diet ↓ number of malformed larvae due to hydrolysate	N.a.	↓ trypsin secretion and AP and lap activity for the 58% diet = enzymatic activities for the 19% diet and diet without hydrolysate	(Cahu et al., 1999)
0.6 mg; 2.1 mg	Commercial enzymatic fish hydrolysate	40%, replacing FM	↑ final weight and survival rates ↓ malformities incidence	N.a.	N.a.	(Cahu et al., 1998)
3 mg; 35 mg	Ultrafiltered enzymatic FM hydrolysate	119 or 237 g kg^{-1}, replacing FM	↑ final weight due to hydrolysate ↑ survival for the 119 g kg^{-1} diet ↓ Malformations with increased hydrolysate inclusion	N.a.	↑ chymotrypsin activity due to hydrolysate ↑ activities of brush border enzymes with age ↓ leu-ala activity with age Earlier enzymatic development in the 119 g kg^{-1} diet	(Infante et al., 1997)
4 mg; 27 mg	Commercial enzymatic hydrolysate	35% hydrolysate in a casein mixture. Mixture	↓ growth and survival for the hydrolysate-only diet compared to live prey	N.a.	↓ trypsin activity in pancreatic segment for the hydrolysate-only diet	(Cahu & Infante, 1995b)

Average fish weight (initial; final), g	Hydrolysate	Inclusion levels	Effects on growth	Effects on nutrient utilisation	Effects on physiology aspects	Ref.
		tested alone or as FM supplement	= growth for FM + hydrolysate diet compared to live prey		↓ intestine trypsin activity due to the hydrolysate ↓ amylase due to hydrolysate = activity of brush border enzymes for the FM + hydrolysate diet and the live prey	
4 mg; 26 mg	Commercial casein hydrolysate	10%, replacing FM	= survival rates to live prey ↓ growth than live prey	N.a.	↓ trypsin activity on pancreatic and intestinal segments due to hydrolysate ↓ amylase activity on pancreatic segment due to hydrolysate = amylase activity on the intestinal segment due to hydrolysate ↑ leu-ala and lap due to hydrolysate = phenylalanine-glycine peptidase. Delayed enterocyte differentiation for compound diets, attenuated by the hydrolysate	(Cahu & Infante, 1995a)
Juveniles						
73.0; 147.1	Enzymatic hydrolysate from salmon heads and backbones	5 or 10%, replacing FM	= SGR, final weight and somatic indices	= FCR, intake, PER. ↑ LER for the 10% diet	↑ plasma cholesterol and potassium in 5% diet ↓ plasma glucose in 10% diet ↓ post-stress (temperature and hypoxia) triglycerides in the 5% diet ↓ lactate due to hydrolysate ↑ *il1β* and *il8* in 10% diet ↑ *il10* in 5% diet	(Parma et al., 2023)
25.8; 70.2	Enzymatic hydrolysed or fermented (or combined processes) brown algae	5%, replacing wheat meal	↓ final weight and SGR for diets with hydrolysed or fermented algae = final weight and SGR for diets with hydrolysed and fermented algae	= feed intake = FCR	↓ intestinal basal transepithelial resistance for all supplemented diets = intestinal permeability to small molecules ↓ intestinal permeability to large molecules for the combined processes algae = most of epithelium integrity-related genes ↓ *cdh17* for all supplemented diets	(Fonseca et al., 2023)

Average fish weight (initial; final), g	Hydrolysate	Inclusion levels	Effects on growth	Effects on nutrient utilisation	Effects on physiology aspects	Ref.
					= most of amino-acid transport- and metabolism-related genes ↓ *slc38a2* for the hydrolysed and fermented and hydrolysed algae ↓ *slc6a16a* for the fermented algae = most immune-related genes ↑ *il6* for the combined process algae = microbial diversity ↑ relative frequency of *Vibrionaceae* in intestinal microbiota	
106.9; 227.2	Fermented and hydrolysed *Ulva rigida* algae	5%, replacing FM	↓ final weight and DGI for the hydrolysate	= feed efficiency, PER, nitrogen and energy retention	= whole-body composition and somatic indices	(Fernandes et al., 2022)
7.0; 30.4	Wild anchovies' hydrolysates (with two different peptidases; A1 and A2) and two squid hydrolysates (with a single peptidase or a mix of peptidases; S2 and SM)	3% in plant-based diet, replacing wheat gluten	↑ final weight and SGR of hydrolysate groups than a plant-based diet, except for S2 = final weight and SGR of hydrolysate groups to a FM-based diet, except for S2	= nutrient intake, nutrient gain and retention	= final whole-body composition, muscle composition, HSI and VSI. ↑ fat content in liver for the SM group. = peroxidase, ACH50 and IgM activities = lysozyme activity for all hydrolysates as a FM-based diet ↑ lysozyme for all hydrolysates than a plant-based diet = haematological parameters	(Costa et al., 2020)
8.3; 17.8 (trial 1) 4.4; 13 (trial 2)	Dry or wet, squid or scallop hydrolysates or a mixture of 60 (scallop)/40 (squid) of both raw materials	50 g kg^{-1} for dry hydrolysates; 282-349 g kg^{-1} for wet hydrolysates (due to variations in moisture); replacing FM	= weight gain for all hydrolysates and FM-based diet	= feed intake. = FCR for all hydrolysates and FM-based diet	↑ feed attractability for all hydrolysates than a low FM diet	(Lee et al., 2018)
10.3; unknown	Commercial enzymatic and spray-dried shrimp hydrolysed	1, 5 or 10% replacing plant proteins	↑ SGR for the 10% diet, above a FM-based diet ↓ SGR for the 1% diet, equal to a low FM diet	↑ feed intake with the increase of hydrolysate level ↓ FCR with the increase of hydrolysate level	Accelerated faeces emission for hydrolysate diets Contractions in perfused intestine due to hydrolysates	(Leduc et al., 2018a)

Average fish weight (initial; final), g	Hydrolysate	Inclusion levels	Effects on growth	Effects on nutrient utilisation	Effects on physiology aspects	Ref.
2.2; 13	Shrimp hydrolysate, tilapia hydrolysate and a 50/50 mix of both	5% in low FM diet	↑ SGR and final weight for all hydrolysates compared to a low FM diet = growth performance between hydrolysates and a high FM diet = condition factor.	= feed intake. = FCR of hydrolysate diets compared to a FM-based diet	↑ intestinal villi height and intestinal globlet cell density for hydrolysates ↑ expression of hydrolases in the tilapia hydrolysate than the shrimp ↓ expression of genes involved in the immune and stimuli response in the tilapia hydrolysate diet than in the shrimp = intestinal transcriptomic profile for hydrolysates and a FM-based diet	(Leduc et al., 2018b)
18.9; 60	Commercial shrimp hydrolysate	5%, replacing vegetable proteins	= survival, final weight and length, SGR, and condition factor, regardless of	↓ FCR for hydrolysates	= fillet proximate composition ↑ lysozyme, ACH50 and bactericidal activity for hydrolysates ↑ survival after an outbreak of *V. Pelagius* for hydrolysates	(Gisbert et al., 2018)
1; 3	Commercial fish hydrolysate; hydrolysed feather meal	Fish: 180 or 300 g kg^{-1}; feather: 313 g kg^{-1}; replacing FM	= final weight and SGR for fish hydrolysate and FM-based diet ↓ growth and SGR for feather hydrolysate	= protein digestibility ↓ energy digestibility for the 180 g kg^{-1} fish hydrolysate = feed intake intake.	= whole-body protein content ↑ RNA to protein ratio for the feather hydrolysate diet ↑ fractional whole-body protein synthesis for the feather hydrolysate diet = fractional whole-body protein synthesis for fish hydrolysate and FM-based diet ↑ protein degradation ratio for the 300 g kg^{-1} fish hydrolysate and feather hydrolysate diets = efficiency of ribosomal activity ↓ efficiency of protein deposition in all hydrolysates	(Langar et al., 1993)

ACH50: alternative haemolytic complement activity; AP: alkaline phosphatase; cdh17 – cadherin-17; DGI – daily growth index; FCR: feed conversion ratio; FM – fishmeal; HSI: hepatosomatic index; IgM: immunoglobulin M; il1β – interleukin 1 β; il6 – interleukin 6; il8 – interleukin 8; il10 – interleukin 10; leu-ala: lap: leucine aminopeptidase N; leucine–alanine peptidase; LER – lipid efficiency ratio; PER – protein efficiency ratio; RNA: ribonucleic acid; SGR – specific growth rate; slc6a16a – solute carrier family 6 member 16a; slc38a2 – solute carrier family 38 member a2; VSI: viscerosomatic index.

These results are utterly important, because with the intensification of aquaculture practices (with high densities and frequent fish manipulation) there has been a rise on the frequency of pathogen infections, with a large impact on both animal welfare (with high mortality rates) and economic balance of aquaculture companies. Additionally, fish farms must avoid the use of antibiotics, to prevent the dissemination of resistant bacteria (European Centre for Disease Prevention and Control et al., 2017), and thus new strategies are necessary to promote fish robustness.

It has been widely demonstrated that hydrolysates rich in BP can promote gut health status in European seabass juveniles, through the enhancement of nutrient transportation and permeability at the intestine, accelerated feed gastrointestinal transit time and increased intestinal villi height (Fonseca et al., 2023; Leduc et al., 2018a; Leduc et al., 2018b). Similarly, in larvae, earlier gut maturation, modulation of pancreatic enzymes, stimulation of brush border enzymes and microbiota modulation have been reported as a consequence of hydrolysate inclusion in feeds (Cahu & Infante, 1995a; Delcroix et al., 2015; Infante et al., 1997; Kotzamanis et al., 2007; Skalli et al., 2014). These results are significant because using high levels of plant ingredients often hinders gut health, by modulating gut microbiota, increasing gut inflammation and altering gut histomorphology (Azeredo et al., 2017b; Serra et al., 2021; Torrecillas et al., 2017b). The restoration of gut health due to hydrolysate inclusion could then result in an improved growth performance of fish fed plant-based diets. Indeed, Costa et al. (2020); Leduc et al. (2018a); Leduc et al. (2018b) and Lee et al. (2018) observed improved growth for European seabass juvenile fed plant-based diets supplemented with hydrolysates, achieving levels similar to those of a FM-based diet.

Although some hydrolysates are reported to possess an antioxidant activity, their potential to ameliorate oxidative stress in European seabass remains underexplored. Furthermore, the possible impact of dietary inclusion of hydrolysates on European seabass response to external stressors such as temperature changes, hypoxia or manipulation is seldom studied. Parma et al. (2023) showed that BPs derived from salmon by-products reduced plasma total protein, cholesterol, high-density lipoprotein and triglycerides of European seabass after environmental stressful conditions (high water temperature and hypoxia). The authors suggest that the hydrolysate led to an enhanced lipid utilization for energy to respond to high energy demand periods.

One aspect to be kept in mind in the development and study of hydrolysates is that variations in both the source and processing significantly influence the resulting ingredients, leading to differences in protein content, AA profile, and generated peptides in the final hydrolysate. This variability accounts for the observed differences in results when using hydrolysates, even when originating from the same source (Costa et al., 2020; Delcroix et al., 2015; Kotzamanis et al., 2007; Skalli et al., 2014).

Overall, hydrolysates rich in BP emerge as interesting ingredients for European seabass diets, particularly at low levels. This suggests their suitability to be incorporated in low-FM diets to address the limitations associated with this dietary approach. However, the effects of hydrolysates are highly variable, underscoring the need for further research to better understand and optimize their impact.

1.4. Functional ingredients – formulating diets towards increased feed intake

1.4.1. Regulation of feed intake in fish

Feed intake modulation is dependent on the interaction of both homeostatic and hedonic signals. The first relates to the animal's necessity to meet energy and nutrient requirements, and the latter is a consequence of pleasant sensations derived from ingesting the feed (Soengas et al., 2018). The hypothalamus and telencephalon play a significant role on integration of both homeostatic and hedonic signals, commanding a complex mechanism that involves numerous central and peripheral tissues and sensory organs (Delgado et al., 2017; Lin et al., 2000). While both olfactive and taste receptors are used by fish to detect food, the final determination of food consumption is mostly validated by taste (Morais, 2017). Taste modalities including umami, bitter and sweet, are mediated by two families of taste receptors, T1Rs and T2Rs, which are G-protein-coupled receptors (Morais, 2017). While T1Rs are associated with the identification of attractive compounds and essential nutrients, T2Rs have been reported to be related to the detection of potentially toxic, harmful or bitter compounds (Morais, 2017). Regarding the olfactive sensory system, the lateral olfactory tract is involved in feeding behaviour, with microvillous sensory neurones that respond to food odours (Hamdani & Døving, 2007). Interaction of food odours and olfactive receptors produces olfactory signals which are then transmitted to the brain, where they are integrated in the feed intake regulation network (Hu et al., 2020). This information is combined with the animals needs for nutrients and energy and ultimately, integration of hedonic and homeostatic signals in the telencephalon and hypothalamus affects their production of specific neuropeptides which directly regulate feed intake. These neuropeptides are the anorexigens (feed intake suppressing) cocaine and amphetamine-related transcript (CART) and pro-opiomelanocortin (POMC), and the orexigens (feed intake stimulating) agouti-related peptide (AgRP) and neuropeptide Y (NPY) (Comesaña et al., 2018; Soengas, 2021).

Regarding the hedonic regulation of feed intake, the opioid system has been reported as being involved (Díaz-Rúa et al., 2022; Soengas et al., 2018). In addition, the endocannabinoid system also plays an important role, promoting overconsumption due to intensification of palatability and orosensory reward of feed, as well as at post-ingestive stages, through interference with ghrelin, leptin and cholecystokinin, which affect the organism capacity to feel satiated (Díaz-Rúa et al., 2020). The dopaminergic system also contributes to goal-directed behaviour, including feeding (Leal et al., 2013). This hedonic regulation may override homeostatic signals, resulting in increased expression of orexigenic molecules and, consequently, feed overconsumption to levels above the minimum to fulfil energy and nutrient requirements (Comesaña et al., 2018; Díaz-Rúa et al., 2022). This can result in an increased fish growth, assuming feed efficiency is not affected.

1.4.2. Feed attractants and stimulants

One problem regarding the replacement of FM and FO with plant ingredients is the impact on diet palatability and, as a consequence, lowered feed intake. Switching from a marine-based diet to a plant-based one led to lower intake on rainbow trout (*Oncorhynchus mykiss*), during the first week of feeding (Geurden et al., 2013). Likewise, Liang et al. (2019) reported anorexia in *Lateolabrax japonicas*, during the first two weeks of feeding, after a total replacement of dietary FM by a plant protein blend. Sabioni et al. (2022) observed that the inclusion of a soy protein concentrate in levels above 50% reduced feed intake in dorado (*Salminus brasilensis*), through the modulation of appetite-regulating hormones. Atlantic salmon (*Salmo salar*) smolts had a reduced intake of a diet containing 80% of plant proteins and 70% vegetable oils, compared to a marine-based aquafeed, after three months of feeding (Torstensen et al., 2008). Feed intake was also reduced in European seabass fed diets devoid of FM (Dias et al., 1997).

While fish may become accustomed to plant-based diets on the long term, which may reduce differences in feed intake, it is important to consider that a temporary decrease in feed consumption, even in the mid-term, followed by habituation, can still affect the overall growth performance of the fish and lead to substantial production losses (Torstensen et al., 2008). In fact, physiological and endocrine functions can be impacted during such habituation periods.

Two concepts should be kept in mind related to feed intake: chemical attraction and feeding stimulation. The first refers to the capacity of feed to attract the animal towards the feed particle and even initiate feeding. In contrast, feed stimulation refers to the ability of feed to affect satiety, which leads to stronger impacts on the total amount of ingested feed. However, many studies do not strictly distinguish these nuances. Table 4 shows the main studies regarding the use of attractants in diets for saltwater species.

Table 4 - Studies reporting evaluation of feed attractants on diets for marine fish species.

Ingredient	Inclusion levels	Fish species	Results on feed intake	Other comments	Ref.
AA mixture	2.5%	European seabass (*D. labrax*)	↑ intake and weight gain for soy-based aquafeeds, to levels similar as those of a FM-based diet	↑ Feed efficiency compared to non-supplemented diets	(Dias et al., 1997)
Jack mackerel meal	1, 3, 5, 20, 20, 40, 60, and 100%	Olive flounder (*Paralichthys olivaceus*)	↑ attractiveness, highest for the 60% diet ↑ feed consumption, highest for the 20% diet ↑ weight gain for the 20, 40 and 60% diets	= Feed efficiency	(Jeong et al., 2022)
Jack mackerel meal, sardine meal, hydrolysed FM	5%	Olive flounder (*P. olivaceus*)	↑ intake for all attractants, maximum for jack mackerel meal ↑ feeding attractiveness for jack mackerel meal, followed by sardine meal and hydrolysed FM	= Feed efficiency ↑ whole-body crude lipid for the jack mackerel and sardine meals Attractants selected from preliminary attractiveness trials, which also included: anchovy meal, herring meal, pollack meal, crab meal, krill meal, shrimp head meal, shrimp meal, oyster meal, squid meal, squid liver meal, corn gluten meal, defatted soya bean meal	(Jeong et al., 2020)
Krill meal	5%	Red Snapper (*Lutjanus campechanus*)	= intake and weight gain as a non-supplemented plant diet ↓ intake and weight gain as a FM-based diet	Fish fed the attractant diet seemed to eat more aggressively than those fed the non-supplemented diet based on observations by the authors	(Walsh et al., 2021)
Jack mackerel meal	1, 3, 5 or 10%	Rockfish (*Sebastes schlegeli*)	↑ intake and weight gain with the growing levels of attractant	= FCR and whole-body proximate composition	(Baek et al., 2021)
AA, quaternary ammonium bases, nucleotides, combination of previous attractants and blue mussel protein hydrolysate	1%	Turbot (*Scophthalmus maximus*)	↓ intake, weight gain and brain *npy* for all attractants compared to a FM-based diet ↑ intake and weight gain for the AA attractant, compared to a non-supplemented diet	= feed efficiency for the AA diet and a FM based diet ↑ intestinal muscular thickness for the AA diet	(Jiang et al., 2019)
Squid meal or squid hydrolysate	1, 2 or 4 g kg^{-1}	Pompano (*Trachinotus carolinus*)	= intake and weight gain as a non-supplemented diet	= FCR	(Novriadi et al., 2017)
Valine, glycine, histidine, combination	3 (valine and histidine), 4 (glycine)	Pompano (*T. carolinus*)	= intake for control diets and diets supplemented with AA	One trial tested the AA as attractants, the other tested the chemical	(Rhodes et al., 2017)

Ingredient	Inclusion levels	Fish species	Results on feed intake	Other comments	Ref.
of the 3 AA or fish protein concentrate; chemical attractant mix or squid hydrolysate	and 10 (fish concentrate) g kg^{-1}; 50 g kg^{-1} (squid hydrolysate and chemical attractant)		↓ weight gain for valine and combination diets ↑ intake and weight gain for the squid hydrolysate	attractant and the squid hydrolysate Chemical attractant from Louisiana State University, Baton Rouge, USA; undisclosed composition	
Blue mussel meal	2, 4 or 8%	Turbot (*Psetta maxima*)	= intake and weight gain compared to the non-supplemented diet ↓ intake compared to a FM-based diet		(Nagel et al., 2014)
FM hydrolysate, polychaete meal, mussel meal, squid meal	10%	Senegalese sole (*Solea senegalensis*)	↑ intake and global acceptability index for the FM hydrolysate = intake for the polychaete meal ↓ intake for the mussel meal and squid meal		(Barroso et al., 2013)
Bivalve flavour or betaine	0.5% (bivalve flavour, for coating); 1.5% (bivalve flavour, for internal inclusion); 3% (betaine, only internal inclusion)	Sole (*Solea solea*)	↑ attractant and arrestant activity for the bivalve flavour externally applied: = stimulant activity for the commercial flavour and betaine.		(Reig et al., 2003)
Antarctic krill hydrolysate; adenosine monophosphate (AMP); choline chloride; AA mix; combination of AMP, choline and AA	19 or 38 g kg^{-1} (krill hydrolysate); 1.5 g kg^{-1} (AMP); 15 g kg^{-1} (choline); 3.2 g kg^{-1} (AA)	Atlantic salmon (*Salmo salar*)	= feed intake, weight gain and brain *npy* and *agrp* ↑ *cart* for the AA diet ↑ *pomc* for the 38 g kg^{-1} krill hydrolysate diet and AA diet	= FCR	(Kousoulaki et al., 2013)
White tea and/or methionine	2.9% (white tea); 0.3% (methionine)	gilthead seabream (*Sparus aurata*)	↑ intake for the methionine diet ↓ intake and weight gain for the white tea diet	=FCR, N and energy retention ↑ Plasma HDL cholesterol and total lipids for the white tea diets ↓ whole-body lipid, plasma glucose, liver glycogen for the white tea diets ↓ liver G6PD, ME and FAS activities	(Pérez-Jiménez et al., 2013)
Betaine	1%	Cobia (*Rachycentron canadum*)	= feed intake and weight gain	↓ FCR in a 0% FM diet supplemented with attractant, compared to the non-supplemented 0% FM	(Trushenski et al., 2011)
Crystalline AA mix; fish soluble; krill meal; squid meal;	2.5% (AA mix); 10% (fish soluble, krill meal or squid meal);	Red seabream (*Pagrus major*)	↑ intake and weight gain for the fish soluble and mixture of meals, compared to both a plant-based and a	↑ Feed efficiency for all supplemented diets, compared to a plant-based diet	(Kader et al., 2010)

Ingredient	Inclusion levels	Fish species	Results on feed intake	Other comments	Ref.
mixture of fish soluble, krill meal and squid meal	15% (mixture of meals)		FM-based diet ↑ intake and weight gain for the AA mix, krill meal and squid meal, compared to a plant-based diet, and = to a FM-based diet		
Squid meal	2.0 g kg^{-1}	Atlantic halibut (*Hippoglossus hippoglossus*)	= feed intake and specific growth rate	= Feed efficiency	(Berge et al., 1999)

AA – amino acids; agrp – agouti-related peptide; AMP – adenosine monophosphate; cart – Cocaine and amphetamine-related transcript; FAS – fatty acid synthetase; FCR: feed conversion ratio; FM – fishmeal; G6PD – Glucose-6-phosphate dehydrogenase; HDL – high-density lipoprotein; IgM: immunoglobulin M; il1β – interleukin 1 β; il8 – interleukin 8; il10 – interleukin 10; leu-ala: lap: leucine aminopeptidase N; leucine–alanine peptidase; LER – lipid efficiency ratio; ME – malic enzyme; npy – neuropeptide Y; PER – protein efficiency ratio; pomc - Pro-opiomelanocortin; RNA: ribonucleic acid; SGR – specific growth rate; slc6a16a – solute carrier family 6 member 16a; slc38a2 – solute carrier family 38 member a2; VSI: viscerosomatic index.

Betaine has been one of the most studied and commonly used feed attractants. It was firstly discovered in sugar beets and it is a zwitterionic compound with a cationic functional group such as a quaternary ammonium or phosphonium, and an anionic functional group such as a carboxylate group (Lim et al., 2022). It was extensively used as attractant for freshwater species (Gule & Geremew, 2022) and it has also been considered for some saltwater species. However, it may not always prove effective in minimizing the reduction of palatability associated with plant-based diets, as reported by Trushenski et al. (2011).

Marine-derived ingredients have also been considered as feed attractants. The AA profile of marine ingredients, which is responsible for the high acceptability of FM-based diets, has led to the suggestion that addition of small amounts of such AA or hydrolysates from marine sources could be used as attractants in less palatable plant-based diets. While the results are mostly favourable, the preferences are often quite species dependent. For example, a squid meal was not effective at increasing intake in diets for Atlantic halibut (*Hippoglossus hippoglossus*) (Berge et al., 1999) or Senegalese sole (*Solea senegalensis*) (Barroso et al., 2013), but it increased intake in red seabream (*Pagrus major*) (Kader et al., 2010). Moreover, differences in the ingredients processing, diet formulation, inclusion levels, among others, may also justify the inconsistent results.

There is still further room for improvement of additives relating to feed intake enhancement. Considering a circular economy approach, marine by-products may be studied as possible feed attractants. However, and while AA and protein-derived meals have been extensively studied, other compounds, including organic acids, aldehydes, alcohols and others are present in several by-products and have been characterized as attractants for vertebrates (Chen et al., 2017; Takács et al., 2018); however, their use in aquafeeds is still underexplored.

1.5. Consumer perception regarding aquaculture products

1.5.1. Consumer priorities at purchase moment

The growth of aquaculture cannot be viable without a deep understanding of the consumer behaviour when purchasing a seafood product. Several factors play a significant role when the consumer purchases a fish or fishery product, including visual cues, perceived quality, price, country of origin, nutritional and health claims and environmental concerns.

Experienced consumers may be capable of assessing the quality of the fresh seafood through its appearance (intrinsic cues), which would then be reflected in a high-quality cooked product. Less experienced consumers might feel difficulties in this evaluation, relying on extrinsic cues, including the price, salesman and labels for quality evaluation. In any case, perceived quality is often claimed as the one of the most, if not the most, important factor when purchasing fish (Masi et al., 2022; Meng et al., 2021; Murray et al., 2017; Zander et al., 2018).

Price plays a major role in the acquisition of food products, and two different perspectives emerge. Some consumer segments are attracted to lower-priced products, while another segment associates higher prices with superior quality and, thus, opts for premium-priced products (Risius et al., 2019).

The country of origin has also been reported as a very important factor in the decision-making process, with consumers often preferring to buy products originating from their own country (Banovic et al., 2019; Nguyen et al., 2015; Risius et al., 2019). This may be an evidence of ethnocentrism (Nguyen et al., 2015) or the perception that local products are fresher and of higher quality compared to those from distant regions (Banovic et al., 2019; Roheim et al., 2012). In labels, the product's country of origin should be clearly displayed to increase consumer's trust in the product.

Fish consumers are aware that consuming these products has a beneficial impact on their health, albeit often confusing the reasons behind that. Verbeke et al. (2005) have found that many consumers mistakenly believe that fish is a good source of dietary fibre and are even unaware of the potential of fish as sources of omega-3 fatty acids and their health benefits. On the other hand, Banovic et al. (2019) showed that nutritional claims (such as "source of omega-3") are favoured to health claims (such as "improved heart function"), and suggest that as consumers already perceive fish as having a beneficial health impact, they do not pay so much attention to such claims.

Ethical consumerism is growing, with increasing consumer awareness across all food sectors. This often leads to a willingness to pay more for products with ethical attributes, regarding their environmental, social and economic impact or their improvement of animal welfare (Feucht & Zander, 2015; Simpson & Radford, 2012). Eco-labels are one way of capturing consumers' attention and showcasing a company's commitment to sustainability. Nevertheless, the emphasis on sustainable and environmentally friendly practices seems to vary depending on culture. Reig et al. (2019) discovered that, in general, Spanish fish consumers did not prioritize this aspect, in contrast to Risius et al. (2019) who demonstrated that a segment of German consumers places greater importance on this issue compared to other factors when buying fish products. Regarding animal welfare, Reig et al. (2019) suggest that this may be important to a niche segment, and Solgaard and Yang (2011) have found that a particular consumer segment may be willing to pay up to 25% more for enhanced fish welfare.

The production method (aquaculture or catches) also influences the consumers' decision when purchasing fishery products, as they may associate it with its perceived quality, sustainability considerations or price. Therefore, this information is mandatory to be included in labels in the EU (European Union, 2013).

The combination of multiple factors affects a consumer's decision to purchase a certain product type, and it is important to explore how consumers perceive the correlations among these attributes with respect to aquaculture products. Such understanding would enable the aquaculture sector to better understand how to enhance the value of its products.

1.5.2. Consumption perception of aquaculture products

With the continuous growth of aquaculture in the last years, consumer perception about these products has become more and more important. Despite this growth, it has been found that consumers still prefer wild seafood over farmed, with farm fish and fishery products sales being below expected (Agência Portuguesa do Ambiente, 2023). As observed in Figure 4, in 2021, both in the European Union and in Portugal, aquaculture production was not able to surpass wild caught seafood, neither in weight nor in volume, according to data from EUMOFA (2023b) and Instituto Nacional de Estatística and Direção-Geral de Recursos Naturais, Segurança e Serviços Marítimos (2022, 2023).

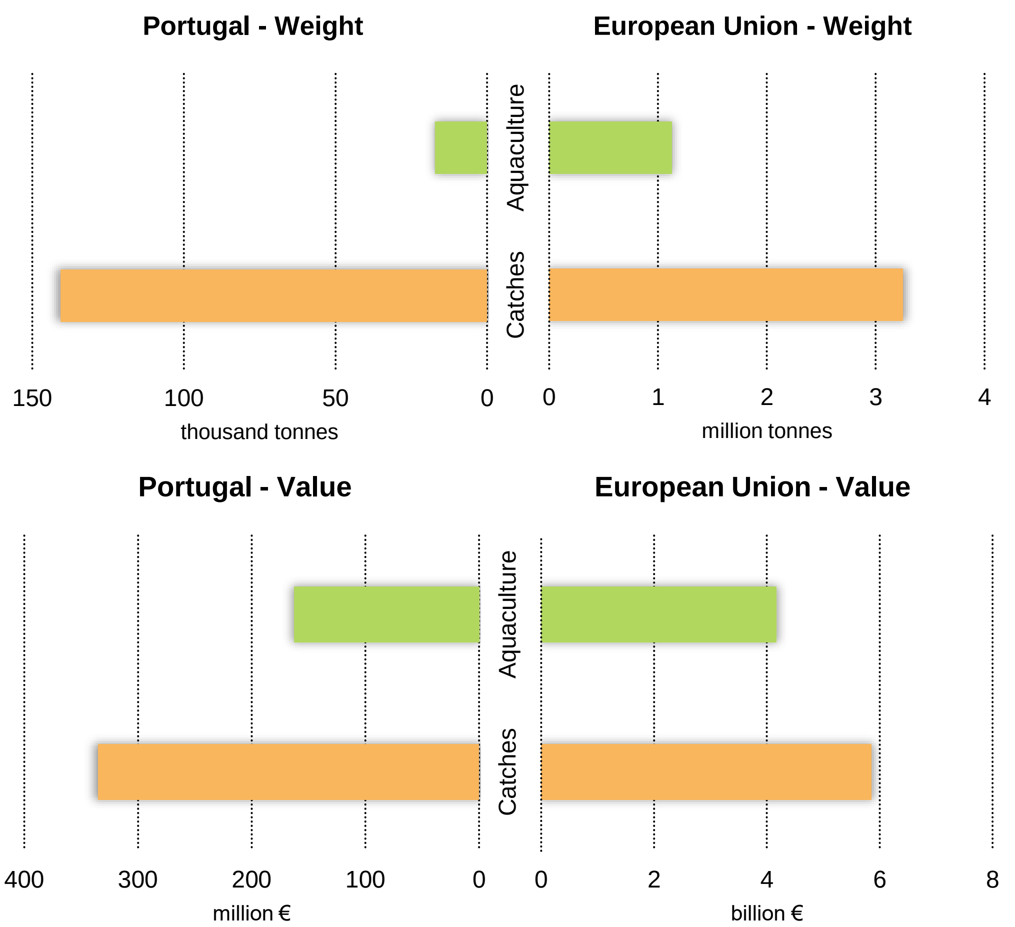

Figure 4 – Production in weight (top) and value (bottom), of aquaculture and catches products, in Portugal (left) and the EU (right) in 2020. Data from EUMOFA (2023b); Instituto Nacional de Estatística and Direção-Geral de Recursos Naturais, Segurança e Serviços Marítimos (2022, 2023).

Data from figure 4 may not represent adequately consumer trends, as it includes fish caught for non-food purposes. However, when analysing the European Union per capita apparent consumption (apparent consumption = imports + aquaculture production + catches – exports; excludes catches for non-food use) of aquaculture and fishery products (including all seafood), it is clear that catches still represent a significant fraction and this has been relatively steady over the last decade (Figure 5).

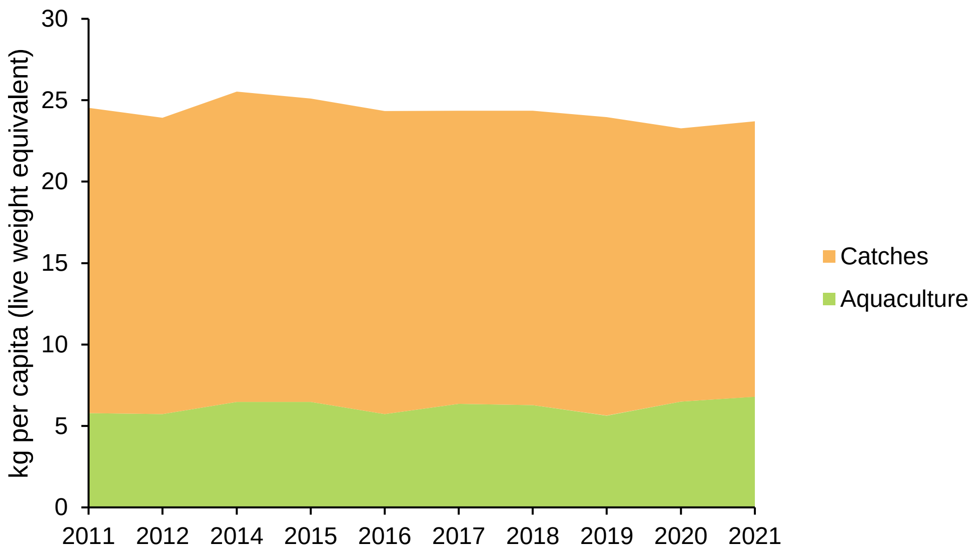

Figure 5 - Per capita apparent consumption of products from aquaculture and catches in the European Union. Data from EUMOFA (2023b).

This European data contrasts with global data by FAO, which shows that 56% of aquatic foods available for consumption are produced by aquaculture. This is mostly due to consumption patterns in Asia, where a higher share of farmed products is consumed (FAO, 2022).

While technological and economic considerations affect the market and its supply capacity, consumers' perception is necessarily a significant driver of this market. In this sense, a survey from the Eurobarometer, about consumer habits regarding fishery and aquaculture products (European Commission, 2021), has revealed that 32% of European Union consumers and 48% of Portuguese consumers still prefer wild-caught seafood products given the choice (Figure 6).

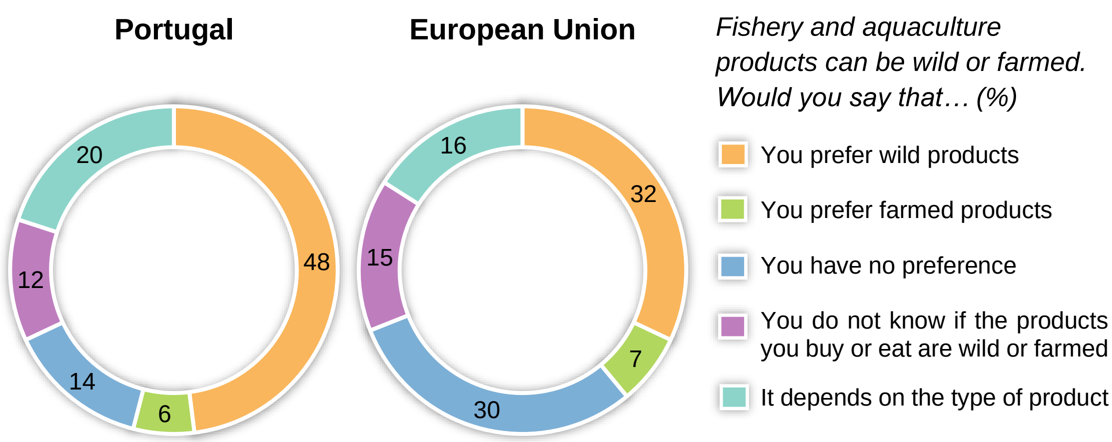

Figure 6 – Preferences of Portuguese (left) and European (right) consumers regarding the origin of fishery and aquaculture products, according to a Eurobarometer survey (European Commission, 2021).

It is imperative to conduct a thorough investigation into the underlying factors driving this preference, as the success of aquaculture is dependent on its perceived image. Moreover, this behaviour can be product-specific and may also vary based on geographical, cultural and temporal contexts, underscoring the need for continuous updates and monitoring to account for such fluctuations.

References

Abdel-Tawwab, M., Khalifa, E., Diab, A. M., Khallaf, M. A., Abdel-Razek, N., & Khalil, R. H. (2020). Dietary garlic and chitosan alleviated zearalenone toxic effects on performance, immunity, and challenge of European sea bass, *Dicentrarchus labrax*, to *Vibrio alginolyticus* infection. *Aquaculture International*, *28*(2), 493-510. https://doi.org/10.1007/s10499-019-00477-0

Abdul Kader, M., Koshio, S., Ishikawa, M., Yokoyama, S., Bulbul, M., Nguyen, B. T., Gao, J., & Laining, A. (2012). Can fermented soybean meal and squid by-product blend be used as fishmeal replacements for Japanese flounder (*Paralichthys olivaceus*)? *Aquaculture Research*, *43*(10), 1427-1438. https://doi.org/10.1111/j.1365-2109.2011.02945.x

Agência Portuguesa do Ambiente. (2023). *Relatório do Estado do Ambiente 2022*. https://rea.apambiente.pt/?language=pt-pt

Aguilar-Toalá, J. E., Hernández-Mendoza, A., González-Córdova, A. F., Vallejo-Cordoba, B., & Liceaga, A. M. (2019). Potential role of natural bioactive peptides for development of cosmeceutical skin products. *Peptides*, *122*(1), 170170. https://doi.org/10.1016/j.peptides.2019.170170

Ahmed, R., & Chun, B.-S. (2018). Subcritical water hydrolysis for the production of bioactive peptides from tuna skin collagen. *The Journal of Supercritical Fluids*, *141*, 88-96. https://doi.org/10.1016/j.supflu.2018.03.006

Akbarian, M., Khani, A., Eghbalpour, S., & Uversky, V. N. (2022). Bioactive peptides: Synthesis, sources, applications, and proposed mechanisms of action. *International Journal of Molecular Sciences*, *23*(3). https://doi.org/10.3390/ijms23031445

Alavi, F., & Ciftci, O. N. (2023). Purification and fractionation of bioactive peptides through membrane filtration: A critical and application review. *Trends in Food Science & Technology*, *131*, 118-128. https://doi.org/10.1016/j.tifs.2022.11.024

Allaoui, A., Barranquero, C., Yahia, S., Herrera-Marcos, L. V., Benomar, S., Jridi, M., Navarro, M. Á., Rodriguez-Yoldi, M. J., Nasri, M., Osada, J., & Boualga, A. (2018). Fenugreek proteins and their hydrolysates prevent hypercholesterolemia and enhance the HDL antioxidant properties in rats. *Nutrition & Food Science*, *48*(6), 973-989. https://doi.org/10.1108/NFS-02-2018-0062

Aloysius, T. A., Tillander, V., Pedrelli, M., Dankel, S. N., Berge, R. K., & Bjørndal, B. (2022). Plasma cholesterol- and body fat-lowering effects of chicken protein hydrolysate and oil in high-fat fed male Wistar rats. *Nutrients*, *14*(24), 5364. https://doi.org/10.3390/nu14245364

Amaral, D., Filipe, D. M., Cavalheri, T., Vieira, L., Magalhães, R. P., Belo, I., Peres, H., & Ozório, R. (2023). Solid-state fermentation of plant feedstuff mixture affected the physiological responses of European seabass (*Dicentrarchus labrax*) reared at different temperatures and subjected to salinity oscillation. *Animals*, *13*(3), 393. https://doi.org/10.3390/ani13030393

Amorim, M., Pinheiro, H., & Pintado, M. (2019). Valorization of spent brewer's yeast: optimization of hydrolysis process towards the generation of stable ACE-inhibitory peptides. *LWT - Food Science and Technology*, *111*(1), 77-84. https://doi.org/10.1016/j.lwt.2019.05.011

Anal, A. K., Noomhorm, A., & Vongsawasdi, P. (2013). Protein Hydrolysates and Bioactive Peptides from Seafood and Crustacean Waste: Their Extraction, Bioactive Properties and Industrial Perspectives. In *Marine Proteins and Peptides* (pp. 709-735). https://doi.org/10.1002/9781118375082.ch36

Anderssen, K. E., & McCarney, E. R. (2020). Online monitoring of enzymatic hydrolysis of marine by-products using benchtop nuclear magnetic resonance spectroscopy. *Food Control*, *112*(1), 107053. https://doi.org/10.1016/j.foodcont.2019.107053

Arru, B., Furesi, R., Gasco, L., Madau, F. A., & Pulina, P. (2019). The introduction of insect meal into fish diet: the first economic analysis on European sea bass farming. *Sustainability*, *11*(6), 1697. https://doi.org/10.3390/su11061697

Aslam, S., Shukat, R., Khan, M. I., & Shahid, M. (2020). Effect of dietary supplementation of bioactive peptides on antioxidant potential of broiler breast meat and physicochemical characteristics of nuggets. *Food Science of Animal Resources*, *40*(1), 55-73. https://doi.org/10.5851/kosfa.2019.e82

Azeredo, R., Machado, M., Afonso, A., Fierro-Castro, C., Reyes-López, F. E., Tort, L., Gesto, M., Conde-Sieira, M., Míguez, J. M., Soengas, J. L., Kreuz, E., Wuertz, S., Peres, H., Oliva-Teles, A., & Costas, B. (2017a). Neuroendocrine and immune responses undertake different fates following tryptophan or methionine dietary treatment: Tales from a teleost model. *Frontiers in Immunology*, *8*. https://doi.org/10.3389/fimmu.2017.01226

Azeredo, R., Machado, M., Fontinha, F., Fernández-Boo, S., Conceição, L. E. C., Dias, J., & Costas, B. (2020). Dietary arginine and citrulline supplementation modulates the immune condition and inflammatory response of European seabass. *Fish & Shellfish Immunology*, *106*. https://doi.org/10.1016/j.fsi.2020.07.060

Azeredo, R., Machado, M., Kreuz, E., Wuertz, S., Oliva-Teles, A., Enes, P., & Costas, B. (2017b). The European seabass (*Dicentrarchus labrax*) innate immunity and gut health are modulated by dietary plant-protein inclusion and prebiotic supplementation. *Fish & Shellfish Immunology*, *60*(1), 78-87. https://doi.org/10.1016/j.fsi.2016.11.019

Baek, S. I., Cho, S. H., & Kim, H. S. (2021). Dietary inclusion effect of various levels of jack mackerel meal on the growth performance, feed efficiency and whole body composition of rockfish (*Sebastes schlegeli*). *Fisheries and Aquatic Sciences*, *24*(9), 311-317. https://doi.org/10.47853/FAS.2021.e30

Baig, M. H., Ahmad, K., Saeed, M., Alharbi, A. M., Barreto, G. E., Ashraf, G. M., & Choi, I. (2018). Peptide based therapeutics and their use for the treatment of neurodegenerative and other diseases. *Biomedicine & Pharmacotherapy*, *103*, 574-581. https://doi.org/10.1016/j.biopha.2018.04.025

Banovic, M., Reinders, M. J., Claret, A., Guerrero, L., & Krystallis, A. (2019). A cross-cultural perspective on impact of health and nutrition claims, country-of-origin and eco-label on consumer choice of new aquaculture products. *Food Research International*, *123*, 36-47. https://doi.org/10.1016/j.foodres.2019.04.031

Barroso, F. G., Rodiles, A., Vizcaino, A. J., Martínez, T. F., & Alarcón, F. J. (2013). Evaluation of feed attractants in juvenile Senegalese sole, *Solea senegalensis*. *Journal of the World Aquaculture Society*, *44*(5), 682-693. https://doi.org/10.1111/jwas.12068

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

Basto, A., Matos, E., & Valente, L. M. P. (2020). Nutritional value of different insect larvae meals as protein sources for European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, *521*, 735085. https://doi.org/10.1016/j.aquaculture.2020.735085

Berge, G. M., Grisdale-Helland, B., & Helland, S. J. (1999). Soy protein concentrate in diets for Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture*, *178*(1), 139-148. https://doi.org/10.1016/S0044-8486(99)00127-1

Bertocci, F., & Mannino, G. (2022). Can agri-food waste be a sustainable alternative in aquaculture? A bibliometric and meta-analytic study on growth performance, innate immune system, and antioxidant defenses. *Foods*, *11*(13), 1861. https://doi.org/10.3390/foods11131861

Bilen, S., Kenanoglu, O. N., Terzi, E., Ozdemir, R. C., & Sonmez, A. Y. (2019). Effects of tetra (*Cotinus coggygria*) and common mallow (*Malva sylvestris*) plant extracts on growth performance and immune response in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). *Aquaculture*, *512*, 734251. https://doi.org/10.1016/j.aquaculture.2019.734251

Bonvini, E., Parma, L., Badiani, A., Fontanillas, R., Gatta, P. P., Sirri, F., Nannoni, E., & Bonaldo, A. (2018). Integrated study on production performance and quality traits of European sea bass (*Dicentrarchus labrax*) fed high plant protein diets. *Aquaculture*, *484*, 126-132. https://doi.org/10.1016/j.aquaculture.2017.10.041

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

Brijs, J., Sandblom, E., Axelsson, M., Sundell, K., Sundh, H., Huyben, D., Broström, R., Kiessling, A., Berg, C., & Gräns, A. (2018). The final countdown: Continuous physiological welfare evaluation of farmed fish during common aquaculture practices before and during harvest. *Aquaculture*, *495*, 903-911. https://doi.org/10.1016/j.aquaculture.2018.06.081

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

Cabillon, N. A. R., & Lazado, C. C. (2019). Mucosal barrier functions of fish under changing environmental conditions. *Fishes*, *4*(1), 2. https://doi.org/10.3390/fishes4010002

Cahu, C., Rønnestad, I., Grangier, V., & Zambonino Infante, J. L. (2004). Expression and activities of pancreatic enzymes in developing sea bass larvae (*Dicentrarchus labrax*) in relation to intact and hydrolyzed dietary protein; involvement of cholecystokinin. *Aquaculture*, *238*(1), 295-308. https://doi.org/10.1016/j.aquaculture.2004.04.013

Cahu, C. L., & Infante, J. L. Z. (1995a). Effect of the molecular form of dietary nitrogen supply in sea bass larvae: Response of pancreatic enzymes and intestinal peptidases. *Fish Physiology and Biochemistry*, *14*(3), 209-214. https://doi.org/10.1007/BF00004311

Cahu, C. L., & Infante, J. L. Z. (1995b). Maturation of the pancreatic and intestinal digestive functions in sea bass (*Dicentrarchus labrax*): Effect of weaning with different protein sources. *Fish Physiology and Biochemistry*, *14*(6), 431-437. https://doi.org/10.1007/BF00004343

Cahu, C. L., Infante, J. L. Z., Escaffre, A. M., Bergot, P., & Kaushik, S. (1998). Preliminary results on sea bass (*Dicentrarchus labrax*) larvae rearing with compound diet from first feeding. Comparison with carp (*Cyprinus carpio*) larvae. *Aquaculture*, *169*(1), 1-7. https://doi.org/10.1016/S0044-8486(98)00316-0

Cahu, C. L., Infante, J. L. Z., Quazuguel, P., & Le Gall, M. M. (1999). Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass *Dicentrarchus labrax* larvae. *Aquaculture*, *171*(1), 109-119. https://doi.org/10.1016/S0044-8486(98)00428-1

Callegaro, K., Brandelli, A., & Daroit, D. J. (2019). Beyond plucking: feathers bioprocessing into valuable protein hydrolysates. *Waste Management*, *95*, 399-415. https://doi.org/10.1016/j.wasman.2019.06.040

Cao, S., Li, P., Huang, B., Song, Z., Hao, T., Wang, C., & Wang, M. (2020). Assessing feasibility of replacement of fishmeal with enzyme-treated feather meal in the diet of juvenile turbot (*Scophthalmus maximus* L.). *Aquaculture Nutrition*, *26*(4), 1340-1352. https://doi.org/10.1111/anu.13090

Casanova-Martí, À., Bravo, F. I., Serrano, J., Ardévol, A., Pinent, M., & Muguerza, B. (2019). Antihyperglycemic effect of a chicken feet hydrolysate via the incretin system: DPP-IV-inhibitory activity and GLP-1 release stimulation. *Food & Function*, *10*(7), 4062-4070. https://doi.org/10.1039/C9FO00695H

Castro, C., Peréz-Jiménez, A., Coutinho, F., Corraze, G., Panserat, S., Peres, H., Teles, A. O., & Enes, P. (2018). Nutritional history does not modulate hepatic oxidative status of European sea bass (*Dicentrarchus labrax*) submitted to handling stress. *Fish Physiology and Biochemistry*, *44*(3), 911-918. https://doi.org/10.1007/s10695-018-0480-6

Ceccotti, C., Al-Sulaivany, B. S. A., Al-Habbib, O. A. M., Saroglia, M., Rimoldi, S., & Terova, G. (2019). Protective effect of dietary taurine from ROS production in European seabass under conditions of forced swimming. *Animals*, *9*(9), 607. https://doi.org/10.3390/ani9090607

Chalamaiah, M., Keskin Ulug, S., Hong, H., & Wu, J. (2019). Regulatory requirements of bioactive peptides (protein hydrolysates) from food proteins. *Journal of Functional Foods*, *58*(1), 123-129. https://doi.org/10.1016/j.jff.2019.04.050

Chen, M., Chen, X., Nsor-Atindana, J., Masamba, K. G., Ma, J., & Zhong, F. (2017). Optimization of key aroma compounds for dog food attractant. *Animal Feed Science and Technology*, *225*, 173-181. https://doi.org/10.1016/j.anifeedsci.2016.12.005

Chung, Y. W., Lee, J. H., Lee, J. Y., Ju, H. H., Lee, Y.-J., Jee, D. H., Ko, S.-H., & A Choi, J. (2020). The anti-inflammatory effects of glucagon-like peptide receptor agonist lixisenatide on the retinal nuclear and nerve fiber layers in an animal model of early type

2 diabetes. *The American Journal of Pathology*, *190*(5), 1080-1094. https://doi.org/10.1016/j.ajpath.2020.01.011

Ciji, A., & Akhtar, M. S. (2021). Stress management in aquaculture: A review of dietary interventions. *Reviews in Aquaculture*, *13*(4), 2190-2247. https://doi.org/10.1111/raq.12565

Comesaña, S., Velasco, C., Conde-Sieira, M., Míguez, J. M., Soengas, J. L., & Morais, S. (2018). Feeding stimulation ability and central effects of intraperitoneal treatment of L-leucine, L-valine, and L-proline on amino acid sensing systems in rainbow trout: Implication in food intake control. *Frontiers in Physiology*, *9*, 1209. https://doi.org/10.3389/fphys.2018.01209

Connolly, A., Piggott, C. O., & FitzGerald, R. J. (2013). Characterisation of protein-rich isolates and antioxidative phenolic extracts from pale and black brewers' spent grain. *International Journal of Food Science & Technology*, *48*(8), 1670-1681. https://doi.org/10.1111/ijfs.12137

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

Council of the European Union. (2006). Council Regulation (EC) No 1198/2006 of 27 July 2006 on the European Fisheries Fund, 1-75. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex:32006R1198

Cruz-Casas, D. E., Aguilar, C. N., Ascacio-Valdés, J. A., Rodríguez-Herrera, R., Chávez-González, M. L., & Flores-Gallegos, A. C. (2023). Bioactive protein hydrolysates obtained from amaranth by fermentation with lactic acid bacteria and *Bacillus* species. *Heliyon*, *9*(2), e13491. https://doi.org/10.1016/j.heliyon.2023.e13491

da Silva, C. P., Bezerra, R. S., dos Santos, A. C. O., Messias, J. B., de Castro, C. R. O. B., & Carvalho Junior, L. B. (2017). Biological value of shrimp protein hydrolysate by-product produced by autolysis. *LWT - Food Science and Technology*, *80*, 456-461. https://doi.org/10.1016/j.lwt.2017.03.008

Dauda, A. B., Ajadi, A., Tola-Fabunmi, A. S., & Akinwole, A. O. (2019). Waste production in aquaculture: sources, components and managements in different culture systems. *Aquaculture and Fisheries*, *4*(3), 81-88. https://doi.org/10.1016/j.aaf.2018.10.002

Dawood, M. A. O., Koshio, S., & Esteban, M. Á. (2018). Beneficial roles of feed additives as immunostimulants in aquaculture: A review. *Reviews in Aquaculture*, *10*(4), 950-974. https://doi.org/10.1111/raq.12209

Delcroix, J., Gatesoupe, F.-J., Desbruyères, E., Huelvan, C., Le Delliou, H., Le Gall, M.-M., Quazuguel, P., Mazurais, D., & Zambonino-Infante, J. L. (2015). The effects of dietary marine protein hydrolysates on the development of sea bass larvae, *Dicentrarchus labrax*, and associated microbiota. *Aquaculture Nutrition*, *21*(1), 98-104. https://doi.org/10.1111/anu.12139

Delgado, M. J., Cerdá-Reverter, J. M., & Soengas, J. L. (2017). Hypothalamic integration of metabolic, endocrine, and circadian signals in fish: involvement in the control of food intake. *Frontiers in Neuroscience*, *11*, 354. https://doi.org/10.3389/fnins.2017.00354

Dias, J., Gomes, E. F., & Kaushik, S. (1997). Improvement of feed intake through supplementation with an attractant mix in European bass fed plant-protein rich diets. *Aquatic Living Resources*, *10*. https://doi.org/10.1051/alr:1997043

Díaz-Rúa, A., Chivite, M., Comesaña, S., Conde-Sieira, M., & Soengas, J. L. (2022). The opioid system in rainbow trout telencephalon is probably involved in the hedonic regulation of food intake. *Frontiers in Physiology*, *13*. https://doi.org/10.3389/fphys.2022.800218

Díaz-Rúa, A., Chivite, M., Comesaña, S., Velasco, C., Valente, L. M. P., Soengas, J. L., & Conde-Sieira, M. (2020). The endocannabinoid system is affected by a high-fat-diet in rainbow trout. *Hormones and Behavior*, *125*, 104825. https://doi.org/10.1016/j.yhbeh.2020.104825

Dinardo, F. R., Maggiolino, A., Casalino, E., Deflorio, M., & Centoducati, G. (2021). A multi-biomarker approach in European sea bass exposed to dynamic temperature changes under dietary supplementation with *Origanum vulgare* essential oil. *Animals*, *11*(4), 982. https://doi.org/10.3390/ani11040982

Ding, Y., Ko, S.-C., Moon, S.-H., & Lee, S.-H. (2019). Protective effects of novel antioxidant peptide purified from alcalase hydrolysate of velvet antler against oxidative stress in Chang liver cells in vitro and in a zebrafish model in vivo. *International Journal of Molecular Sciences*, *20*(20), 5187. https://doi.org/10.3390/ijms20205187

Dixon, B., van Erp, S. H. M., Rodrigues, P. N. S., Egberts, E., & Stet, R. M. (1995). Fish major histocompatibility complex genes: An expansion. *Developmental & Comparative Immunology*, *19*(2), 109-133. https://doi.org/10.1016/0145-305X(94)00056-L

Echave, J., Fraga-Corral, M., Garcia-Perez, P., Popović-Djordjević, J., H. Avdović, E., Radulović, M., Xiao, J., A. Prieto, M., & Simal-Gandara, J. (2021). Seaweed protein hydrolysates and bioactive peptides: Extraction, purification, and applications. *Marine Drugs*, *19*(9), 500. https://doi.org/10.3390/md19090500

EFSA Panel on Dietetic Products Nutrition and Allergies. (2021). Scientific and technical guidance for the preparation and presentation of a dossier for evaluation of an infant and/or follow-on formula manufactured from protein hydrolysates (Revision 1) 1. *EFSA Journal*, *19*(3), e06556. https://doi.org/10.2903/j.efsa.2021.6556

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), 4194. https://doi.org/10.1038/s41598-020-60325-7

Enes, P., Panserat, S., Kaushik, S., & Oliva-Teles, A. (2011). Dietary carbohydrate utilization by European sea bass (*Dicentrarchus labrax* L.) and gilthead sea bream (*Sparus aurata* L.) juveniles. *Reviews in Fisheries Science*, *19*(3), 201-215. https://doi.org/10.1080/10641262.2011.579363

EUMOFA. (2023a). *Aquaculture data - Yearly simple tables*. Retrieved on March 28, 2023 from https://www.eumofa.eu/en/aquaculture-yearly

EUMOFA. (2023b). *The EU Fish Market*. https://doi.org/10.2771/38507

European Centre for Disease Prevention and Control, European Food Safety Authority, & European Medicines Agency. (2017). *ECDC, EFSA and EMA Joint Scientific Opinion on a list of outcome indicators as regards surveillance of antimicrobial resistance and antimicrobial consumption in humans and food-producing animals*. https://www.ecdc.europa.eu/sites/default/files/documents/AMR-indicators-joint-report-Oct-2017.pdf

European Commission. (2021). *Special Eurobarometer 515 - EU Consumer Habits Regarding Fishery and Aquaculture Products*. 10.2771/87688

European Union. (2003). Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. https://eur-lex.europa.eu/eli/reg/2003/1831/2021-03-27

European Union. (2013). Regulation (EU) No 1379/2013 of the European Parliament and of the Council of 11 December 2013 on the common organisation of the markets in fishery and aquaculture products, amending Council Regulations (EC) No 1184/2006 and (EC) No 1224/2009 and repealing Council Regulation (EC) No 104/2000. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32013R1379

European Union. (2019). Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC https://eur-lex.europa.eu/eli/reg/2019/6/oj

Fan, X., Hu, S., Wang, K., Yang, R., & Zhang, X. (2020). Coupling of ultrasound and subcritical water for peptides production from *Spirulina platensis*. *Food & Bioproducts Processing*, *121*, 105-112. https://doi.org/10.1016/j.fbp.2020.01.012

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

Faustino, M., Veiga, M., Sousa, P., Costa, E. M., Silva, S., & Pintado, M. (2019). Agro-food byproducts as a new source of natural food additives. *Molecules*, *24*(6), 1056. https://doi.org/10.3390/molecules24061056

Fernandes, H., Martins, N., Vieira, L., Salgado, J. M., Castro, C., Oliva-Teles, A., Belo, I., & Peres, H. (2022). Pre-treatment of *Ulva rigida* improves its nutritional value for European seabass (*Dicentrarchus labrax*) juveniles. *Algal Research*, *66*, 102803. https://doi.org/10.1016/j.algal.2022.102803

Ferreira, M., Machado, M., Mota, C. S. C., Abreu, H., Silva, J., Maia, M. R. G., Kiron, V., Costas, B., & Valente, L. M. P. (2023). Micro- and macroalgae blend modulates the mucosal and systemic immune responses of European seabass (*Dicentrarchus labrax*) upon infection with *Tenacibaculum maritimum*. *Aquaculture*, *566*, 739222. https://doi.org/10.1016/j.aquaculture.2022.739222

Feucht, Y., & Zander, K. (2015). Of earth ponds, flow-through and closed recirculation systems - German consumers' understanding of sustainable aquaculture and its communication. *Aquaculture*, *438*, 151-158. https://doi.org/10.1016/j.aquaculture.2015.01.005

Fleurence, J., Morançais, M., Dumay, J., Decottignies, P., Turpin, V., Munier, M., Garcia-Bueno, N., & Jaouen, P. (2012). What are the prospects for using seaweed in human nutrition and for marine animals raised through aquaculture? *Trends in Food Science & Technology*, *27*(1), 57-61. https://doi.org/10.1016/j.tifs.2012.03.004

Fonseca, F., Fuentes, J., Vizcaíno, A. J., Alarcón, F. J., Mancera, J. M., Martínez-Rodríguez, G., & Martos-Sitcha, J. A. (2023). From invasion to fish fodder: Inclusion of the brown algae *Rugulopteryx okamurae* in aquafeeds for European sea bass *Dicentrarchus labrax* (L., 1758). *Aquaculture*, *568*, 739318. https://doi.org/10.1016/j.aquaculture.2023.739318

Francis, D. S., & Turchini, G. M. (2017). Retro-engineering the protein sparing effect to preserve n-3 LC-PUFA from catabolism and optimise fish oil utilisation: A preliminary case study on juvenile Atlantic salmon. *Aquaculture*, *468*, 184-192. https://doi.org/10.1016/j.aquaculture.2016.10.013

Fu, R., Liang, C., Chen, D., Tian, G., Zheng, P., He, J., Yu, J., Mao, X., Luo, Y., Luo, J., & Yu, B. (2023). Yeast hydrolysate attenuates lipopolysaccharide-induced inflammatory responses and intestinal barrier damage in weaned piglets. *Journal of*

Animal Science and Biotechnology, *14*(1), 44. https://doi.org/10.1186/s40104-023-00835-2

Fu, Y., Bak, K. H., Liu, J., De Gobba, C., Tøstesen, M., Hansen, E. T., Petersen, M. A., Ruiz-Carrascal, J., Bredie, W. L. P., & Lametsch, R. (2019). Protein hydrolysates of porcine hemoglobin and blood: Peptide characteristics in relation to taste attributes and formation of volatile compounds. *Food Research International*, *121*, 28-38. https://doi.org/10.1016/j.foodres.2019.03.017

Galindo-Villegas, J., & Mulero, V. (2014). Current knowledge on the development and functionality of immune responses in the European sea bass (*Dicentrarchus labrax*). In F. J. S. Vázquez & J. A. Muñoz-Cueto (Eds.), *Biology of European Sea Bass* (pp. 342-373). CRC Press. https://doi.org/10.1201/b16043-14

Gan, J., Xiao, Z., Wang, K., Kong, X., Du, M., Wang, Z., Xu, B., & Cheng, Y. (2023). Isolation, characterization, and molecular docking analyses of novel calcium-chelating peptide from soy yogurt and the study of its calcium chelation mechanism. *Journal of the Science of Food and Agriculture*, *103*(6), 2939-2948. https://doi.org/10.1002/jsfa.12370

Gao, R., Yu, Q., Shen, Y., Chu, Q., Chen, G., Fen, S., Yang, M., Yuan, L., McClements, D. J., & Sun, Q. (2021). Production, bioactive properties, and potential applications of fish protein hydrolysates: Developments and challenges. *Trends in Food Science & Technology*, *110*, 687-699. https://doi.org/10.1016/j.tifs.2021.02.031

Geurden, I., Borchert, P., Balasubramanian, M. N., Schrama, J. W., Dupont-Nivet, M., Quillet, E., Kaushik, S. J., Panserat, S., & Médale, F. (2013). The positive impact of the early-feeding of a plant-based diet on its future acceptance and utilisation in rainbow trout. *PLOS ONE*, *8*(12), e83162. https://doi.org/10.1371/journal.pone.0083162

Gisbert, E., Fernández, I., Villamizar, N., Dias, M. J., Zambonino-Infante, J. L., & Estévez, A. (2014). European Sea Bass Larval Culture. In F. J. S. Vázquez & J. A. Muñoz-Cueto (Eds.), *Biology of European Sea Bass* (pp. 162-207). CRC Press.

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

Gonçalves, R. A., Serradeiro, R., Machado, M., Costas, B., Hunger, C., & Dias, J. (2019). Interactive effects of dietary fishmeal level and plant essential oils supplementation on European sea bass, *Dicentrarchus labrax*: Growth performance, nutrient utilization, and immunological response. *Journal of the World Aquaculture Society*, *50*(6), 1078-1092. https://doi.org/10.1111/jwas.12616

Gourtay, C., Chabot, D., Audet, C., Madec, L., Huelvan, C., Ducros, L., Claireaux, G., Mazurais, D., & Zambonino-Infante, J.-L. (2020). Effect of thermal and nutritional conditions on fatty acid metabolism and oxidative stress response in juvenile European sea bass (*Dicentrarchus labrax*). *Marine Biology*, *167*(10), 144. https://doi.org/10.1007/s00227-020-03729-3

Gule, T. T., & Geremew, A. (2022). Dietary strategies for better utilization of aquafeeds in tilapia farming. *Aquaculture Nutrition*, *2022*, 9463307. https://doi.org/10.1155/2022/9463307

Guo, Y., Wang, K., Wu, B., Wu, P., Duan, Y., & Ma, H. (2020). Production of ACE inhibitory peptides from corn germ meal by an enzymatic membrane reactor with a novel gradient diafiltration feeding working-mode and *in vivo* evaluation of antihypertensive effect. *Journal of Functional Foods*, *64*, 103584. https://doi.org/10.1016/j.jff.2019.103584

Güroy, B., Güroy, D., Bilen, S., Kenanoğlu, O. N., Şahin, I., Terzi, E., Karadal, O., & Mantoğlu, S. (2022). Effect of dietary Spirulina (*Arthrospira platensis*) on the growth performance, immune-related gene expression and resistance to *Vibrio anguillarum* in European seabass (*Dicentrarchus labrax*). *Aquaculture Research*, *53*(6), 2263-2274. https://doi.org/10.1111/are.15745

Güroy, D., Şahin, İ., Güroy, B., Merrifield, D. L., Bulut, M., & Tekinay, A. A. (2013). Replacement of fishmeal with rice protein concentrate in practical diets for European sea bass *Dicentrarchus labrax* reared at winter temperatures. *Aquaculture Research*, *44*(3), 462-471. https://doi.org/10.1111/j.1365-2109.2011.03053.x

Hamdani, E. H., & Døving, K. B. (2007). The functional organization of the fish olfactory system. *Progress in Neurobiology*, *82*(2), 80-86. https://doi.org/10.1016/j.pneurobio.2007.02.007

Harnedy, P. A., Parthsarathy, V., McLaughlin, C. M., O'Keeffe, M. B., Allsopp, P. J., McSorley, E. M., O'Harte, F. P. M., & FitzGerald, R. J. (2018). Blue whiting (*Micromesistius poutassou*) muscle protein hydrolysate with *in vitro* and *in vivo* antidiabetic properties. *Journal of Functional Foods*, *40*, 137-145. https://doi.org/10.1016/j.jff.2017.10.045

Helal, A., Nasuti, C., Sola, L., Sassi, G., Tagliazucchi, D., & Solieri, L. (2023). Impact of spontaneous fermentation and inoculum with natural whey starter on peptidomic profile and biological activities of cheese whey: A comparative study. *Fermentation*, *9*(3), 270. https://doi.org/10.3390/fermentation9030270

Hernández-Corroto, E., Plaza, M., Marina, M. L., & García, M. C. (2020). Sustainable extraction of proteins and bioactive substances from pomegranate peel (*Punica granatum L.*) using pressurized liquids and deep eutectic solvents. *Innovative Food Science & Emerging Technologies*, *60*, 102314. https://doi.org/10.1016/j.ifset.2020.102314

Hou, C.-Y., Hazeena, S. H., Hsieh, S.-L., Ciou, J.-Y., Hsieh, C.-W., Shih, M.-K., Chen, M.-H., Tu, C.-W., & Huang, P.-H. (2023). Investigation of the optimal production conditions for egg white hydrolysates and physicochemical characteristics. *Journal of Food Science and Technology*, *60*(5), 1600-1611. https://doi.org/10.1007/s13197-023-05708-0

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), 24. https://doi.org/10.1186/s40104-017-0153-9

Hu, J., Le, Q., Wang, Y., Kuang, S., Zhang, M., Gu, W., Sun, Y., Jacques, K. J., Li, Y., Zhang, Y., Sun, J., Yang, Y., Xu, S., & Yan, X. (2020). Comparative transcriptome analysis of olfactory epithelium in large yellow croaker: Evidence for olfactory adaptation to feed phagostimulant in fish. *Aquaculture*, *519*, 734920. https://doi.org/10.1016/j.aquaculture.2020.734920

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

Infante, J. L. Z., Cahu, C. L., & Peres, A. (1997). Partial substitution of di- and tripeptides for native proteins in sea bass diet improves *Dicentrarchus labrax* larval development. *The Journal of Nutrition*, *127*(4), 608-614. https://doi.org/10.1093/jn/127.4.608

Instituto Nacional de Estatística, & Direção-Geral de Recursos Naturais, Segurança e Serviços Marítimos. (2022). *Estatísticas da Pesca 2021*. https://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_destaques&DESTAQUESdest_boui=539423546&DESTAQUESmodo=2

Instituto Nacional de Estatística, & Direção-Geral de Recursos Naturais, Segurança e Serviços Marítimos. (2023). *Estatísticas da Pesca 2022*. https://www.dgrm.mm.gov.pt/esta

Islam, M. J., Kunzmann, A., Henjes, J., & Slater, M. J. (2021). Can dietary manipulation mitigate extreme warm stress in fish? The case of European seabass, *Dicentrarchus labrax*. *Aquaculture*, *545*, 737153. https://doi.org/10.1016/j.aquaculture.2021.737153

Jannathulla, R., Rajaram, V., Kalanjiam, R., Ambasankar, K., Muralidhar, M., & Dayal, J. S. (2019). Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. *Aquaculture Research*, *50*(12), 3493-3506. https://doi.org/10.1111/are.14324

Jeong, H. S., Choi, D. G., Lee, K. W., Cho, S. H., Lim, S. G., Lee, B. J., Hur, S. W., Son, M. H., Lee, S. H., & Kim, K. W. (2020). Attractiveness of various crude feed ingredients to juvenile olive flounder (*Paralichthys olivaceus*, Temminck & Schlegel) and its application to aquaculture. *Aquaculture Research*, *51*(11), 4517-4532. https://doi.org/10.1111/are.14797

Jeong, H. S., Kim, J., Olowe, O. S., & Cho, S. H. (2022). Dietary optimum inclusion level of jack mackerel meal for olive flounder (*Paralichthys olivaceus*, Temminck & Schlegel, 1846). *Aquaculture*, *559*, 738432. https://doi.org/10.1016/j.aquaculture.2022.738432

Jiang, D., Zheng, J., Dan, Z., Tang, Z., Ai, Q., & Mai, K. (2019). Effects of five compound attractants in high plant-based diets on feed intake and growth performance of juvenile turbot (*Scophthalmus maximus* L.). *Aquaculture Research*, *50*(9), 2350-2358. https://doi.org/10.1111/are.14116

Kader, M. A., Koshio, S., Ishikawa, M., Yokoyama, S., & Bulbul, M. (2010). Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, *Pagrus major*. *Aquaculture*, *308*(3), 136-144. https://doi.org/10.1016/j.aquaculture.2010.07.037

Kaushik, S. J., Gouillou-Coustans, M. F., & Cho, C. Y. (1998). Application of the recommendations on vitamin requirements of finfish by NRC (1993) to salmonids and sea bass using practical and purified diets. *Aquaculture*, *161*(1), 463-474. https://doi.org/10.1016/S0044-8486(97)00293-7

Khan, A. I., Rehman, A. U., Farooqui, N. A., Siddiqui, Nimra Z., Ayub, Q., Ramzan, M. N., Zexu, W., Zhang, X., Yu, Y., Xin, Y., & Wang, L. (2022). Shrimp peptide hydrolysate modulates the immune response in cyclophosphamide immunosuppressed mice model. *Journal of Food Biochemistry*, *46*(9), e14251. https://doi.org/10.1111/jfbc.14251

Koide, H., Saito, K., Yoshimatsu, K., Chou, B., Hoshino, Y., Yonezawa, S., Oku, N., Asai, T., & Shea, K. J. (2023). Cooling-induced, localized release of cytotoxic peptides from engineered polymer nanoparticles in living mice for cancer therapy. *Journal of Controlled Release*, *355*, 745-759. https://doi.org/10.1016/j.jconrel.2023.02.020

Kokou, F., & Fountoulaki, E. (2018). Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. *Aquaculture*, *495*, 295-310. https://doi.org/10.1016/j.aquaculture.2018.06.003

Kotzamanis, Y. P., Gisbert, E., Gatesoupe, F. J., Zambonino Infante, J., & 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 & Integrative Physiology, *147*(1), 205-214. https://doi.org/10.1016/j.cbpa.2006.12.037

Kousoulaki, K., Rønnestad, I., Olsen, H. J., Rathore, R., Campbell, P., Nordrum, S., Berge, R. K., Mjøs, S. A., Kalananthan, T., & Albrektsen, S. (2013). Krill hydrolysate free amino acids responsible for feed intake stimulation in Atlantic salmon (*Salmo salar*). *Aquaculture Nutrition*, *19*(s1), 47-61. https://doi.org/10.1111/anu.12094

Kousoulaki, K., Sæther, B.-S., Albrektsen, S., & Noble, C. (2015). Review on European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758) nutrition and feed management: A practical guide for optimizing feed formulation and farming protocols. *Aquaculture Nutrition*, *21*(2), 129-151. https://doi.org/10.1111/anu.12233

Krogdahl, Bakke-Mckellep, RØed, & Baeverfjord. (2000). Feeding Atlantic salmon *Salmo salar* L. soybean products: effects on disease resistance (furunculosis), and lysozyme and IgM levels in the intestinal mucosa. *Aquaculture Nutrition*, *6*(2), 77-84. https://doi.org/10.1046/j.1365-2095.2000.00129.x

Kumar, V., & Barman, D. (2012). Anti-nutritional factors in plant feedstuffs used in aquafeeds. *World Aquaculture 43*(3), 64-68.

Kumari, R., Sharma, N., Sharma, S., Samurailatpam, S., Padhi, S., Singh, S. P., & Kumar Rai, A. (2023). Production and characterization of bioactive peptides in fermented soybean meal produced using proteolytic *Bacillus* species isolated from kinema. *Food Chemistry*, *421*, 136130. https://doi.org/10.1016/j.foodchem.2023.136130

Kurniadi, N., Yasni, S., Budijanto, S., & Boing Sitanggang, A. (2023). Continuous production of velvet bean-based bioactive peptides in membrane reactor with dual enzyme system. *Food Chemistry*, *423*, 136378. https://doi.org/10.1016/j.foodchem.2023.136378

Lall, S. P. (2003). The Minerals. In J. E. Halver & R. W. Hardy (Eds.), *Fish Nutrition* (pp. 259-308). Academic Press. https://doi.org/10.1016/B978-012319652-1/50006-9

Lall, S. P., & Kaushik, S. J. (2021). Nutrition and metabolism of minerals in fish. *Animals*, *11*(9), 2711. https://doi.org/10.3390/ani11092711

Langar, H., Guillaume, J., Metailler, R., & Fauconneau, B. (1993). Augmentation of protein synthesis and degradation by poor dietary amino acid balance in European sea bass (*Dicentrarchus labrax*). *The Journal of Nutrition*, *123*(10), 1754-1761. https://doi.org/10.1093/jn/123.10.1754

Leal, E., Fernández-Durán, B., Agulleiro, M. J., Conde-Siera, M., Míguez, J. M., & Cerdá-Reverter, J. M. (2013). Effects of dopaminergic system activation on feeding behavior and growth performance of the sea bass (*Dicentrarchus labrax*): A self-feeding approach. *Hormones and Behavior*, *64*(1), 113-121. https://doi.org/10.1016/j.yhbeh.2013.05.008

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), 396. https://doi.org/10.1186/s12864-018-4780-0

Lee, C. M., Volson, B., Kang, B., Karayannakidis, P. D., Gamez, E., Miller, J., Betty, G., Andrikos, C., Attwater, A., & Bengtson, D. A. (2018). Effects of dietary scallop and squid hydrolysates on growth of European seabass, *Dicentrarchus labrax*; California yellowtail, *Seriola lalandi*; and barramundi, *Lates calcarifer*. *Journal of the World Aquaculture Society*, *49*(6), 971-984. https://doi.org/10.1111/jwas.12572

Li-Chan, E. C. Y. (2015). Bioactive peptides and protein hydrolysates: Research trends and challenges for application as nutraceuticals and functional food ingredients. *Current Opinion in Food Science*, *1*, 28-37. https://doi.org/10.1016/j.cofs.2014.09.005

Li, D., Wang, G., Du, L., Zheng, Y., & Wang, Z. (2022). Recent advances in intelligent recognition methods for fish stress behavior. *Aquacultural Engineering*, *96*, 102222. https://doi.org/10.1016/j.aquaeng.2021.102222

Li, M., Zhang, Y., Xia, S., & Ding, X. (2019). Finding and isolation of novel peptides with anti-proliferation ability of hepatocellular carcinoma cells from mung bean protein hydrolysates. *Journal of Functional Foods*, *62*, 103557. https://doi.org/10.1016/j.jff.2019.103557

Liang, X., Han, J., Xue, M., Yu, H., Huang, H., Wu, X., Zheng, Y., Qin, Y., & Liang, X. (2019). Growth and feed intake regulation responses to anorexia, adaptation and fasting in Japanese seabss, *Lateolabrax japonicas* when fishmeal is totally replaced by plant protein. *Aquaculture*, *498*, 528-538. https://doi.org/10.1016/j.aquaculture.2018.09.010

Lim, L.-S., Liew, K.-S., Ebi, I., Shapawi, R., Mohamad Lal, M. T., Liew, H. J., Hamasaki, K., Masuda, R., & Kawamura, G. (2022). Amino acids as chemoattractant and feeding stimulant for the commercially farmed decapod crustaceans: A brief review. *Aquaculture Research*, *53*(2), 333-343. https://doi.org/10.1111/are.15591

Lin, J., Comi, M., Vera, P., Alessandro, A., Qiu, K., Wang, J., Wu, S.-g., Qi, G.-h., & Zhang, H.-j. (2023). Effects of *Saccharomyces cerevisiae* hydrolysate on growth performance, immunity function, and intestinal health in broilers. *Poultry Science*, *102*(1), 102237. https://doi.org/10.1016/j.psj.2022.102237

Lin, L., Zhu, Q., Zheng, L., Zhao, M., Fan, J., & Liu, S. (2020). Preparation of sea cucumber (*Stichopus variegates*) peptide fraction with desired organoleptic property and its anti-aging activity in fruit flies and D-galactose-induced aging mice. *Journal of Functional Foods*, *69*, 103954. https://doi.org/10.1016/j.jff.2020.103954

Lin, X., Volkoff, H., Narnaware, Y., Bernier, N. J., Peyon, P., & Peter, R. E. (2000). Brain regulation of feeding behavior and food intake in fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *126*(4), 415-434. https://doi.org/10.1016/S1095-6433(00)00230-0

Liu, L., Li, S., Zheng, J., Bu, T., He, G., & Wu, J. (2020). Safety considerations on food protein-derived bioactive peptides. *Trends in Food Science & Technology*, *96*, 199-207. https://doi.org/10.1016/j.tifs.2019.12.022

Llorente, I., Fernández-Polanco, J., Baraibar-Diez, E., Odriozola, M. D., Bjørndal, T., Asche, F., Guillen, J., Avdelas, L., Nielsen, R., Cozzolino, M., Luna, M., Fernández-Sánchez, J. L., Luna, L., Aguilera, C., & Basurco, B. (2020). Assessment of the economic performance of the seabream and seabass aquaculture industry in the European Union. *Marine Policy*, *117*, 103876. https://doi.org/10.1016/j.marpol.2020.103876

Maage, A., Hove, H., & Julshamn, K. (2008). Monitoring and surveillance to improve farmed fish safety. In Ø. Lie (Ed.), *Improving Farmed Fish Quality and Safety* (pp. 547-564). Woodhead Publishing. https://doi.org/10.1533/9781845694920.3.547

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

Machado, M., Peixoto, D., Santos, P., Ricardo, A., Duarte, I., Carvalho, I., Aragão, C., Azeredo, R., & Costas, B. (2022). Tryptophan modulatory role in European seabass (*Dicentrarchus labrax*) immune response to acute inflammation under stressful conditions. *International Journal of Molecular Sciences*, *23*(20), 12475. https://doi.org/10.3390/ijms232012475

Magnadottir, B. (2010). Immunological control of fish diseases. *Marine Biotechnology*, *12*(4), 361-379. https://doi.org/10.1007/s10126-010-9279-x

Malcorps, W., Kok, B., van't Land, M., Fritz, M., van Doren, D., Servin, K., van der Heijden, P., Palmer, R., Auchterlonie, N. A., Rietkerk, M., Santos, M. J., & Davies, S. J.

(2019). The sustainability conundrum of fishmeal substitution by plant ingredients in shrimp feeds. *Sustainability*, *11*(4), 1212. https://doi.org/10.3390/su11041212

Manzanarez-Quin, C. G., García-Romo, J. S., Beltrán-Barrientos, L. M., Torres-Llanez, M. J., Mazorra-Manzano, M. A., Hernández-Mendoza, A., González-Córdova, A. F., & Vallejo-Cordoba, B. (2023). Novel peptides in fermented milk with specific *Lactobacillus* strains potential antiobesity effect: *in vitro* and *in silico* analysis. *ACS Food Science & Technology*, *3*(3), 428-438. https://doi.org/10.1021/acsfoodscitech.2c00347

Marcet, I., Álvarez, C., Paredes, B., & Díaz, M. (2014). Inert and oxidative subcritical water hydrolysis of insoluble egg yolk granular protein, functional properties, and comparison to enzymatic hydrolysis. *Journal of Agricultural & Food Chemistry*, *62*(32), 8179-8186. https://doi.org/10.1021/jf405575c

Markets and Markets. (2022). *Feed Additives Market by Livestock, Type (Phosphates, Amino Acids, Vitamins, Acidifiers, Carotenoids, Enzymes, Flavors & Sweeteners, Mycotoxins Detoxifiers, Minerals, and Antioxidants), Form, Source and Region - Global Forecast to 2028*. Retrieved on 14 Nov 2023 from https://www.marketsandmarkets.com/Market-Reports/feed-additives-market-870.html

Masi, M., Di Pasquale, J., Vecchio, Y., Pauselli, G., Tribilustova, E., & Adinolfi, F. (2022). A cross-sectional study in Mediterranean European countries to support stakeholders in addressing future market demands: Consumption of farmed fish products. *Aquaculture Reports*, *24*, 101133, Article 101133. https://doi.org/10.1016/j.aqrep.2022.101133

Maulu, S., Hasimuna, O. J., Haambiya, L. H., Monde, C., Musuka, C. G., Makorwa, T. H., Munganga, B. P., Phiri, K. J., & Nsekanabo, J. D. (2021). Climate change effects on aquaculture production: sustainability implications, mitigation, and adaptations. *Frontiers in Sustainable Food Systems*, *5*. https://doi.org/10.3389/fsufs.2021.609097

Meng, T., Wang, C., Florkowski, W. J., & Yang, Z. (2021). Determinants of urban consumer expenditure on aquatic products in Shanghai, China. *Aquaculture Economics and Management*, *27*(1), 1-24. https://doi.org/10.1080/13657305.2021.1996480

Mirzaei, M., Mirdamadi, S., Ehsani, M. R., Aminlari, M., & Hosseini, E. (2015). Purification and identification of antioxidant and ACE-inhibitory peptide from *Saccharomyces cerevisiae* protein hydrolysate. *Journal of Functional Foods*, *19*(A), 259-268. https://doi.org/10.1016/j.jff.2015.09.031

Monzón-Atienza, L., Bravo, J., Fernández-Montero, Á., Charlie-Silva, I., Montero, D., Ramos-Vivas, J., Galindo-Villegas, J., & Acosta, F. (2022). Dietary supplementation of *Bacillus velezensis* improves *Vibrio anguillarum* clearance in European sea bass by activating essential innate immune mechanisms. *Fish & Shellfish Immunology*, *124*, 244-253. https://doi.org/10.1016/j.fsi.2022.03.032

Morais, S. (2017). The physiology of taste in fish: Potential implications for feeding stimulation and gut chemical sensing. *Reviews in Fisheries Science & Aquaculture*, *25*(2), 133-149. https://doi.org/10.1080/23308249.2016.1249279

Murray, G., Wolff, K., & Patterson, M. (2017). Why eat fish? Factors influencing seafood consumer choices in British Columbia, Canada. *Ocean and Coastal Management*, *144*, 16-22. https://doi.org/10.1016/j.ocecoaman.2017.04.007

Nagel, F., von Danwitz, A., Schlachter, M., Kroeckel, S., Wagner, C., & Schulz, C. (2014). Blue mussel meal as feed attractant in rapeseed protein-based diets for turbot (*Psetta maxima* L.). *Aquaculture Research*, *45*(12), 1964-1978. https://doi.org/10.1111/are.12140

Nagodawithana, T. W., Nelles, L., & Trivedi, N. B. (2008). Protein hydrolysates as hypoallergenic, flavors and palatants for companion animals. In A. Demain & V. Pasupuleti (Eds.), *Protein Hydrolysates in Biotechnology* (pp. 191-207). Springer. https://doi.org/10.1007/978-1-4020-6674-0_11

Nastić, N., Švarc-Gajić, J., Delerue-Matos, C., Barroso, M. F., Soares, C., Moreira, M. M., Morais, S., Mašković, P., Gaurina Srček, V., Slivac, I., Radošević, K., & Radojković, M. (2018). Subcritical water extraction as an environmentally-friendly technique to recover bioactive compounds from traditional Serbian medicinal plants. *Industrial Crops and Products*, *111*, 579-589. https://doi.org/10.1016/j.indcrop.2017.11.015

National Research Council. (2011). *Nutrient requirements of fish and shrimp*. The National Academies Press. https://doi.org/10.17226/13039

Naylor, R. L., Hardy, R. W., Bureau, D. P., Chiu, A., Elliott, M., Farrell, A. P., Forster, I., Gatlin, D. M., Goldburg, R. J., Hua, K., & Nichols, P. D. (2009). Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences*, *106*(36), 15103. https://doi.org/10.1073/pnas.0905235106

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*, 551-563. https://doi.org/10.1038/s41586-021-03308-6

Nguyen, T. T., Haider, W., Solgaard, H. S., Ravn-Jonsen, L., & Roth, E. (2015). Consumer willingness to pay for quality attributes of fresh seafood: A labeled latent class model. *Food Quality and Preference*, *41*, 225-236. https://doi.org/10.1016/j.foodqual.2014.12.007

Novriadi, R., Spangler, E., Rhodes, M., Hanson, T., & Allen Davis, D. (2017). Effects of various levels of squid hydrolysate and squid meal supplementation with enzyme-treated soy on growth performance, body composition, serum biochemistry and histology of Florida pompano *Trachinotus carolinus*. *Aquaculture*, *481*, 85-93. https://doi.org/10.1016/j.aquaculture.2017.08.032

Oliva-Teles, A., & Pimentel-Rodrigues, A. (2004). Phosphorus requirement of European sea bass (*Dicentrarchus labrax* L.) juveniles. *Aquaculture Research*, *35*(7), 636-642. https://doi.org/10.1111/j.1365-2109.2004.01059.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

Pelletier, N., Klinger, D. H., Sims, N. A., Yoshioka, J.-R., & Kittinger, J. N. (2018). Nutritional attributes, substitutability, scalability, and environmental intensity of an illustrative subset of current and future protein sources for aquaculture feeds: Joint consideration of potential synergies and trade-offs. *Environmental Science & Technology*, *52*(10), 5532-5544. https://doi.org/10.1021/acs.est.7b05468

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

Peres, H., & Oliva-Teles, A. (1999). Effect of dietary lipid level on growth performance and feed utilization by European sea bass juveniles (*Dicentrarchus labrax*). *Aquaculture*, *179*(1), 325-334. https://doi.org/10.1016/S0044-8486(99)00168-4

Peres, H., & Oliva-Teles, A. (2002). Utilization of raw and gelatinized starch by European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, *205*(3), 287-299. https://doi.org/10.1016/S0044-8486(01)00682-2

Peres, H., & Oliva-Teles, A. (2006). Effect of the dietary essential to non-essential amino acid ratio on growth, feed utilization and nitrogen metabolism of European sea bass (*Dicentrarchus labrax*). *Aquaculture*, *256*(1), 395-402. https://doi.org/10.1016/j.aquaculture.2006.02.010

Peres, H., & Oliva-Teles, A. (2007). Effect of the dietary essential amino acid pattern on growth, feed utilization and nitrogen metabolism of European sea bass (*Dicentrarchus labrax*). *Aquaculture*, *267*(1), 119-128. https://doi.org/10.1016/j.aquaculture.2007.01.010

Pérez-Jiménez, A., Peres, H., Rubio, V. C., & Oliva-Teles, A. (2013). Effects of diet supplementation with white tea and methionine on lipid metabolism of gilthead sea bream juveniles (*Sparus aurata*). *Fish Physiology and Biochemistry*, *39*(3), 661-670. https://doi.org/10.1007/s10695-012-9728-8

Prabha, J., Nithin, A., Mariarose, L., & Vincent, S. (2020). Processing of nutritive fish protein hydrolysate from *Leiognathus splendens*. *International Journal of Peptide Research and Therapeutics*, *26*(2), 861-871. https://doi.org/10.1007/s10989-019-09892-6

Prem, R., & Tewari, V. K. (2020). Development of human-powered fish feeding machine for freshwater aquaculture farms of developing countries. *Aquacultural Engineering*, *88*, 102028. https://doi.org/10.1016/j.aquaeng.2019.102028

Raa, J. (1996). The use of immunostimulatory substances in fish and shellfish farming. *Reviews in Fisheries Science*, *4*(3), 229-288. https://doi.org/10.1080/10641269609388587

Reig, L., Escobar, C., Carrassón, M., Constenla, M., Gil, J. M., Padrós, F., Piferrer, F., & Flos, R. (2019). Aquaculture perceptions in the Barcelona metropolitan area from fish and seafood wholesalers, fishmongers, and consumers. *Aquaculture*, *510*, 256-266. https://doi.org/10.1016/j.aquaculture.2019.05.066

Reig, L., Ginovart, M., & Flos, R. (2003). Modification of the feeding behaviour of sole (*Solea solea*) through the addition of a commercial flavour as an alternative to betaine. *Aquatic Living Resources*, *16*(4), 370-379. https://doi.org/10.1016/S0990-7440(03)00062-7

Rhodes, M. A., Zhou, Y., Salze, G. P., Hanson, T. R., & Davis, D. A. (2017). Development of plant-based diets and the evaluation of dietary attractants for juvenile Florida pompano, *Trachinotus carolinus* L. *Aquaculture Nutrition*, *23*(5), 1065-1075. https://doi.org/10.1111/anu.12474

Ringø, E., Van Doan, H., Lee, S. H., Soltani, M., Hoseinifar, S. H., Harikrishnan, R., & Song, S. K. (2020). Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture. *Journal of Applied Microbiology*, *129*(1), 116-136. https://doi.org/10.1111/jam.14628

Risius, A., Hamm, U., & Janssen, M. (2019). Target groups for fish from aquaculture: Consumer segmentation based on sustainability attributes and country of origin. *Aquaculture*, *499*, 341-347. https://doi.org/10.1016/j.aquaculture.2018.09.044

Rivas-Vela, C. I., Castaño-Tostado, E., Cardador-Martínez, A., Amaya-Llano, S. L., & Castillo-Herrera, G. A. (2023). Subcritical water hydrolysis for the obtention of bioactive peptides from a grasshopper *Sphenarium purpurascens* protein concentrate. *The Journal of Supercritical Fluids*, *197*, 105893. https://doi.org/10.1016/j.supflu.2023.105893

Rivero-Ramírez, F., Torrecillas, S., Betancor, M. B., Izquierdo, M. S., Caballero, M. J., & Montero, D. (2020). Effects of dietary arachidonic acid in European sea bass

(*Dicentrarchus labrax*) distal intestine lipid classes and gut health. *Fish Physiology and Biochemistry*, *46*(2), 681-697. https://doi.org/10.1007/s10695-019-00744-0

Roheim, C. A., Sudhakaran, P. O., & Durham, C. A. (2012). Certification of shrimp and salmon for best aquaculture practices: Assessing consumer preferences in Rhode Island. *Aquaculture Economics and Management*, *16*(3), 266-286. https://doi.org/10.1080/13657305.2012.713075

Rojo-Cebreros, A. H., Ibarra-Castro, L., & Martínez-Brown, J. M. (2018). Immunostimulation and trained immunity in marine fish larvae. *Fish & Shellfish Immunology*, *80*, 15-21. https://doi.org/10.1016/j.fsi.2018.05.044

Sabioni, R. E., Lorenz, E. K., Cyrino, J. E. P., & Volkoff, H. (2022). Feed intake and gene expression of appetite-regulating hormones in *Salminus brasiliensis* fed diets containing soy protein concentrate. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *268*, 111208. https://doi.org/10.1016/j.cbpa.2022.111208

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

Saleh, N., A. Wassef, E., & M. Shalaby, S. (2018). The role of dietary astaxanthin in European sea bass (*Dicentrarchus labrax*) growth, immunity, antioxidant competence and stress tolerance. *Egyptian Journal of Aquatic Biology and Fisheries*, *22*(5), 189-200. https://doi.org/10.21608/ejabf.2018.21044

Samaranayaka, A. G. P., & Li-Chan, E. C. Y. (2008). Autolysis-assisted production of fish protein hydrolysates with antioxidant properties from Pacific hake (*Merluccius productus*). *Food Chemistry*, *107*(2), 768-776. https://doi.org/10.1016/j.foodchem.2007.08.076

Schreck, C. (2000). Accumulation and long-term effects of stress in fish. In G. P. Moberg & J. A. Mench (Eds.), *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare* (pp. 147–158). CAB International. https://doi.org/10.1079/9780851993591.0147

Schreck, C. B., & Tort, L. (2016). The concept of stress in fish. In C. B. Schreck, L. Tort, A. P. Farrell, & C. J. Brauner (Eds.), *Fish Physiology* (Vol. 35, pp. 1-34). Academic Press. https://doi.org/10.1016/B978-0-12-802728-8.00001-1

Serra, C. R., Oliva-Teles, A., Enes, P., & Tavares, F. (2021). Gut microbiota dynamics in carnivorous European seabass (*Dicentrarchus labrax*) fed plant-based diets. *Scientific Reports*, *11*(1), 447. https://doi.org/10.1038/s41598-020-80138-y

Serradell, A., Montero, D., Fernández-Montero, Á., Terova, G., Makol, A., Valdenegro, V., Acosta, F., Izquierdo, M. S., & Torrecillas, S. (2022). Gill oxidative stress protection through the use of phytogenics and galactomannan oligosaccharides as functional additives in practical diets for European sea bass (*Dicentrarchus labrax*) juveniles. *Animals*, *12*(23), 3332. https://doi.org/10.3390/ani12233332

Serradell, A., Torrecillas, S., Makol, A., Valdenegro, V., Fernández-Montero, A., Acosta, F., Izquierdo, M. S., & Montero, D. (2020). Prebiotics and phytogenics functional additives in low fish meal and fish oil based diets for European sea bass (*Dicentrarchus labrax*): Effects on stress and immune responses. *Fish & Shellfish Immunology*, *100*, 219-229. https://doi.org/10.1016/j.fsi.2020.03.016

Shen, C., Lin, Y., Mohammadi, T. N., Masuda, Y., Honjoh, K.-i., & Miyamoto, T. (2022). Characterization of novel antimicrobial peptides designed on the basis of amino acid sequence of peptides from egg white hydrolysate. *International Journal of Food Microbiology*, *378*, 109802. https://doi.org/10.1016/j.ijfoodmicro.2022.109802

Siddik, M. A. B., Pham, H. D., Francis, D. S., Vo, B. V., & Shahjahan, M. (2021). 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*(S1), 86-98. https://doi.org/10.1111/anu.13404

Silva-Brito, F., Timóteo, F., Esteves, Â., Peixoto, M. J., Ozorio, R., & Magnoni, L. (2019). Impact of the replacement of dietary fish oil by animal fats and environmental salinity on the metabolic response of European Seabass (*Dicentrarchus labrax*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *233*, 46-59. https://doi.org/10.1016/j.cbpb.2019.04.004

Simpson, B. J. K., & Radford, S. K. (2012). Consumer perceptions of sustainability: A free elicitation study. *Journal of Nonprofit & Public Sector Marketing*, *24*(4), 272-291. https://doi.org/10.1080/10495142.2012.733654

Skalli, A., Infante, J. L. Z., Kotzamanis, Y., Fabregat, R., & Gisbert, E. (2014). Peptide molecular weight distribution of soluble protein fraction affects growth performance and quality in European sea bass (*Dicentrarchus labrax*) larvae. *Aquaculture Nutrition*, *20*(2), 118-131. https://doi.org/10.1111/anu.12058

Skalli, A., & Robin, J. H. (2004). Requirement of n-3 long chain polyunsaturated fatty acids for European sea bass (*Dicentrarchus labrax*) juveniles: Growth and fatty acid composition. *Aquaculture*, *240*(1), 399-415. https://doi.org/10.1016/j.aquaculture.2004.06.036

Soengas, J. L. (2021). Integration of nutrient sensing in fish hypothalamus. *Frontiers in Neuroscience*, *15*, 653928. https://doi.org/10.3389/fnins.2021.653928

Soengas, J. L., Cerdá-Reverter, J. M., & Delgado, M. J. (2018). Central regulation of food intake in fish: an evolutionary perspective. *Journal of Molecular Endocrinology*, *60*(4), R171-R199. https://doi.org/10.1530/jme-17-0320

Solgaard, H. S., & Yang, Y. (2011). Consumers' perception of farmed fish and willingness to pay for fish welfare. *British Food Journal*, *113*(8), 997-1010. https://doi.org/10.1108/00070701111153751

Stafford, J. L., & Belosevic, M. (2003). Transferrin and the innate immune response of fish: identification of a novel mechanism of macrophage activation. *Developmental & Comparative Immunology*, *27*(6), 539-554. https://doi.org/10.1016/S0145-305X(02)00138-6

Suárez, S., Aphalo, P., Rinaldi, G., Añón, M. C., & Quiroga, A. (2020). Effect of amaranth proteins on the RAS system. In vitro, in vivo and ex vivo assays. *Food Chemistry*, *308*, 125601. https://doi.org/10.1016/j.foodchem.2019.125601

Sun, M., Hassan, S. G., & Li, D. (2016). Models for estimating feed intake in aquaculture: A review. *Computers and Electronics in Agriculture*, *127*, 425-438. https://doi.org/10.1016/j.compag.2016.06.024

Sun, X., Ruan, S., Zhuang, Y., & Sun, L. (2021). Anti-osteoporosis effect and purification of peptides with high calcium-binding capacity from walnut protein hydrolysates. *Food & Function*, *12*(18), 8454-8466. https://doi.org/10.1039/D1FO01094H

Swain, P., & Nayak, S. K. (2009). Role of maternally derived immunity in fish. *Fish & Shellfish Immunology*, *27*(2), 89-99. https://doi.org/10.1016/j.fsi.2009.04.008

Tacias-Pascacio, V. G., Morellon-Sterling, R., Siar, E.-H., Tavano, O., Berenguer-Murcia, Á., & Fernandez-Lafuente, R. (2020). Use of alcalase in the production of bioactive peptides: A review. *International Journal of Biological Macromolecules*, *165*, 2143-2196. https://doi.org/10.1016/j.ijbiomac.2020.10.060

Tai, H.-M., Huang, H.-N., Tsai, T.-Y., You, M.-F., Wu, H.-Y., Rajanbabu, V., Chang, H.-Y., Pan, C.-Y., & Chen, J.-Y. (2020). Dietary supplementation of recombinant antimicrobial peptide *Epinephelus lanceolatus* piscidin improves growth performance and immune response in *Gallus gallus domesticus*. *PLOS ONE*, *15*(3), e0230021. https://doi.org/10.1371/journal.pone.0230021

Takács, S., Musso, A. E., Gries, R., Rozenberg, E., Borden, J. H., Brodie, B., & Gries, G. (2018). New food baits for trapping house mice, black rats and brown rats. *Applied Animal Behaviour Science*, *200*, 130-135. https://doi.org/10.1016/j.applanim.2017.11.011

Tocher, D. R. (2010). Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research*, *41*(5), 717-732. https://doi.org/10.1111/j.1365-2109.2008.02150.x

Toldrá, F., Gallego, M., Reig, M., Aristoy, M.-C., & Mora, L. (2020). Recent progress in enzymatic release of peptides in foods of animal origin and assessment of bioactivity.

Journal of Agricultural and Food Chemistry, *68*(46), 12842-12855. https://doi.org/10.1021/acs.jafc.9b08297

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

Torrecillas, S., Mompel, D., Caballero, M. J., Montero, D., Merrifield, D., Rodiles, A., Robaina, L., Zamorano, M. J., Karalazos, V., Kaushik, S., & Izquierdo, M. (2017b). Effect of fishmeal and fish oil replacement by vegetable meals and oils on gut health of European sea bass (*Dicentrarchus labrax*). *Aquaculture*, *468*, 386-398. https://doi.org/10.1016/j.aquaculture.2016.11.005

Torrecillas, S., Rivero-Ramírez, F., Izquierdo, M. S., Caballero, M. J., Makol, A., Suarez-Bregua, P., Fernández-Montero, A., Rotllant, J., & Montero, D. (2018). Feeding European sea bass (*Dicentrarchus labrax*) juveniles with a functional synbiotic additive (mannan oligosaccharides and *Pediococcus acidilactici*): An effective tool to reduce low fishmeal and fish oil gut health effects? *Fish & Shellfish Immunology*, *81*, 10-20. https://doi.org/10.1016/j.fsi.2018.07.007

Torrecillas, S., Terova, G., Makol, A., Serradell, A., Valdenegro, V., Gini, E., Izquierdo, M., Acosta, F., & Montero, D. (2019). Dietary phytogenics and galactomannan oligosaccharides in low fish meal and fish oil-based diets for European sea bass (*Dicentrarchus labrax*) juveniles: Effects on gut health and implications on in vivo gut bacterial translocation. *PLOS ONE*, *14*(9), e0222063. https://doi.org/10.1371/journal.pone.0222063

Torstensen, B. E., Espe, M., Sanden, M., Stubhaug, I., Waagbø, R., Hemre, G. I., Fontanillas, R., Nordgarden, U., Hevrøy, E. M., Olsvik, P., & Berntssen, M. H. G. (2008). Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. *Aquaculture*, *285*(1), 193-200. https://doi.org/10.1016/j.aquaculture.2008.08.025

Trushenski, J., Laporte, J., Lewis, H., Schwarz, M., Delbos, B., Takeuchi, R., & Sampaio, L. A. (2011). Fish meal replacement with soy-derived protein in feeds for juvenile cobia: Influence of replacement level and attractant supplementation. *Journal of the World Aquaculture Society*, *42*(3), 435-443. https://doi.org/10.1111/j.1749-7345.2011.00484.x

Ulug, S. K., Jahandideh, F., & Wu, J. (2021). Novel technologies for the production of bioactive peptides. *Trends in Food Science & Technology*, *108*, 27-39. https://doi.org/10.1016/j.tifs.2020.12.002

Urbizo-Reyes, U., San Martin-González, M. F., Garcia-Bravo, J., López Malo Vigil, A., & Liceaga, A. M. (2019). Physicochemical characteristics of chia seed (*Salvia hispanica*) protein hydrolysates produced using ultrasonication followed by microwave-assisted hydrolysis. *Food Hydrocolloids*, *97*, 105187. https://doi.org/10.1016/j.foodhyd.2019.105187

Valente, L. M. P., Batista, S., Ribeiro, C., Pereira, R., Oliveira, B., Garrido, I., Baião, L. F., Tulli, F., Messina, M., Pierre, R., Abreu, H., Pintado, M., & Kiron, V. (2021). Physical processing or supplementation of feeds with phytogenic compounds, alginate oligosaccharide or nucleotides as methods to improve the utilization of *Gracilaria gracilis* by juvenile European seabass (*Dicentrarchus labrax*). *Aquaculture*, *530*, 735914. https://doi.org/10.1016/j.aquaculture.2020.735914

Vanhonacker, F., Pieniak, Z., & Verbeke, W. (2013). European consumer image of farmed fish, wild fish, seabass and seabream. *Aquaculture International*, *21*(5), 1017-1033. https://doi.org/10.1007/s10499-012-9609-2

Verbeke, W., Sioen, I., Pieniak, Z., Van Camp, J., & De Henauw, S. (2005). Consumer perception versus scientific evidence about health benefits and safety risks from fish consumption. *Public Health Nutrition*, *8*(4), 422-429. https://doi.org/10.1079/PHN2004697

Voss, G. B., Osorio, H., Valente, L. M. P., & Pintado, M. E. (2019). Impact of thermal treatment and hydrolysis by Alcalase and *Cynara cardunculus* enzymes on the functional and nutritional value of Okara. *Process Biochemistry*, *83*, 137-147. https://doi.org/10.1016/j.procbio.2019.05.010

Wael, A., & Hiroyuki, Y. (2013). Production of amino and organic acids from protein using sub-critical water technology. *International Journal of Chemical Reactor Engineering*, *11*(1), 369-384. https://doi.org/10.1515/ijcre-2013-0017

Walsh, S., Davis, R., Weldon, A., Reis, J., Stites, W., Rhodes, M., Ibarra-Castro, L., Bruce, T., & Davis, D. A. (2021). Effects of fishmeal replacement, attractants, and taurine removal on juvenile and sub-adult Red Snapper (*Lutjanus campechanus*). *Aquaculture*, *544*, 737054. https://doi.org/10.1016/j.aquaculture.2021.737054

Wan, A. H. L., Davies, S. J., Soler-Vila, A., Fitzgerald, R., & Johnson, M. P. (2019). Macroalgae as a sustainable aquafeed ingredient. *Reviews in Aquaculture*, *11*(3), 458-492. https://doi.org/10.1111/raq.12241

Wang, A. R., Ran, C., Ringø, E., & Zhou, Z. G. (2018). Progress in fish gastrointestinal microbiota research. *Reviews in Aquaculture*, *10*(3), 626-640. https://doi.org/10.1111/raq.12191

Wubshet, S. G., Lindberg, D., Veiseth-Kent, E., Kristoffersen, K. A., Böcker, U., Washburn, K. E., & Afseth, N. K. (2019). Bioanalytical aspects in enzymatic protein hydrolysis of by-products. In C. M. Galanakis (Ed.), *Proteins: Sustainable Source, Processing and Applications* (pp. 225-258). Academic Press. https://doi.org/10.1016/B978-0-12-816695-6.00008-8

Yazaki, M., Ito, Y., Yamada, M., Goulas, S., Teramoto, S., Nakaya, M.-a., Ohno, S., & Yamaguchi, K. (2017). Oral ingestion of collagen hydrolysate leads to the transportation of highly concentrated Gly-Pro-Hyp and its hydrolyzed form of Pro-Hyp into the bloodstream and skin. *Journal of Agricultural and Food Chemistry*, *65*(11), 2315-2322. https://doi.org/10.1021/acs.jafc.6b05679

Zambonino-Infante, J. L., Panserat, S., Servili, A., Mouchel, O., Madec, L., & Mazurais, D. (2019). Nutritional programming by dietary carbohydrates in European sea bass larvae: Not always what expected at juvenile stage. *Aquaculture*, *501*, 441-447. https://doi.org/10.1016/j.aquaculture.2018.11.056

Zander, K., Risius, A., Feucht, Y., Janssen, M., & Hamm, U. (2018). Sustainable aquaculture products: Implications of consumer awareness and of consumer preferences for promising market communication in Germany. *Journal of Aquatic Food Product Technology*, *27*(1), 5-20. https://doi.org/10.1080/10498850.2017.1390028

Zhang, K., Li, J., Hou, H., Zhang, H., & Li, B. (2019). Purification and characterization of a novel calcium-biding decapeptide from Pacific cod (*Gadus Macrocephalus*) bone: Molecular properties and calcium chelating modes. *Journal of Functional Foods*, *52*, 670-679. https://doi.org/10.1016/j.jff.2018.11.042

Zhang, Q., Jing, X., Zhao, Y., Xia, D., Liu, S., Li, D., Hao, Q., Wang, M., Yu, Z., Li, S., Ran, C., Yang, Y., Zhang, Z., & Zhou, Z. (2023). Partial replacement of pelleted feed by moist fermented feed improved the feed conversion efficiency, liver and intestine health, and gut microbiota structure in common carp (*Cyprinus carpio*). *Aquaculture Reports*, *32*, 101690. https://doi.org/10.1016/j.aqrep.2023.101690

Zhanzhan, L., Bing, Z., Ning, W., Zhenqiang, Z., Hong, W., & Fangqing, Z. (2023). A novel peptide protects against diet-induced obesity by suppressing appetite and modulating the gut microbiota. *Gut*, *72*(4), 686. https://doi.org/10.1136/gutjnl-2022-328035

Zhao, B., Cui, Y., Fan, X., Qi, P., Liu, C., Zhou, X., & Zhang, X. (2019). Anti-obesity effects of *Spirulina platensis* protein hydrolysate by modulating brain-liver axis in high-fat diet fed mice. *PLOS ONE*, *14*(6), e0218543. https://doi.org/10.1371/journal.pone.0218543

Zou, J., & Secombes, C. J. (2016). The function of fish cytokines. *Biology*, *5*(2), 23. https://doi.org/10.3390/biology5020023

2. THESIS MAIN OBJECTIVES

The main goal of this thesis is to contribute to a sustainable growth of aquaculture. This is achieved with a holistic perspective. Not only must we fully comprehend the consumers' preferences regarding aquaculture products, but also promote aquaculture growth minimizing its economic and environmental impacts. Thus, this project aims at using sustainable ingredients to incorporate in fish diets, considering a circular economy approach and the "zero waste" policy. These sustainable ingredients can be found in several by-products of the agri-food industry, and their incorporation may not only contribute to a higher sustainability of aquaculture practices, but also to adding value to such by-products. Finally, the consumer perception for farmed fish will be assessed, and strategies for improving the general acceptance of such products will be addressed. In more detail, the following objectives are to be reached:

- Characterization of different hydrolysed swine blood fractions and their inclusion as sources of bioactive peptides in feeds for European seabass;
- Understanding the effect of hydrolysed swine blood fractions on fish response to stress factors (e.g. exposure to a pathogenic agent or manipulation);
- Recovery of aromas from canning industry wastes and assessment of their potential to modulate feed intake, at short and long term, of European seabass;
- Evaluation of the impact of aroma incorporation on fish fillets quality for human consumption;
- Collecting knowledge regarding the acceptance of farmed fish and proposal of strategies able to build consumer's trust and improve valorisation of the product.

3. Blood hydrolysates as ingredients for functional diets for European seabass

3.1. Innovative swine blood hydrolysates as promising ingredients for European seabass diets: Impact on growth performance and resistance to *Tenacibaculum maritimum* infection

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Innovative swine blood hydrolysates as promising ingredients for European seabass diets: Impact on growth performance and resistance to *Tenacibaculum maritimum* infection

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A R T I C L E I N F O

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A B S T R A C T

Hydrolysates from high-protein coproducts are rich in bioactive peptides (small amino acid chains with properties beyond their nutritional value). When included in aquafeeds, they may promote fish robustness while increasing animal blood value and minimizing waste, contributing to a circular economy. This work assessed how innovatively produced blood hydrolysates (BH) included in plant-based diets for European seabass (*Dicentrarchus labrax*) affected growth performance and resistance to *Tenacibaculum maritimum* infection. Three fractions of swine BH obtained by autohydrolysis (AH) or enzymatically were selected. AH fraction was oven-dried, whilst the enzymatically obtained BH were further submitted to a micro (RMF) and nanofiltration (RNF) and the respective retentates freeze-dried. A commercially based diet was formulated, where 50% of fishmeal was replaced by vegetable proteins, and used as negative control (NC). Each BH fraction was then added at 3% to the NC and further compared to a fishmeal-based diet (positive control, PC); all diets remained isolipidic and isoproteic. Diets were assigned to triplicate groups of European seabass juveniles (12.3 ± 1.4 g) and fed three times daily until apparent satiation in a recirculating saltwater system. After a 12-week growth trial, fish were bath challenged with *T. maritimum* and cumulative mortality followed for 8 days. A digestibility trial was also performed in parallel. Hydrolysates-containing diets had a significantly lower apparent digestibility coefficient (ADC) of dry matter compared to the PC, but RNF did not differ from the NC. RMF and AH had lower lipid ADCs than both controls, while the RNF only differed from the PC. Mineral ADCs of PC, NC and RNF were similar. Calcium ADC in RMF and AH was significantly lower than all other diets; RMF also displayed lower copper and potassium ADCs. Fish fed PC had the highest final weight, followed by NC and RNF. Daily growth index and feed conversion ratio (FCR) of RNF was similar to both controls. RMF induced the lowest final weight, highest FCR and reduced *villus* length in anterior intestine. This diet has also resulted in increased plasmatic peroxidase, but lower lysozyme, compared to all other treatments, showing the lowest disease resistance. Results clearly show that the RNF was the only test diet that significantly reduced European seabass mortality after *T. maritimum* infection, without affecting fish growth in relation to a practical plant-protein based diet, highlighting its high potential for aquafeeds supplementation.

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1. Introduction

The replacement of marine-based ingredients such as fishmeal (FM) and fish oil for farmed marine carnivorous species has been thoroughly investigated, as these ingredients become more and more scarce, expensive and environmentally unsustainable (Naylor et al., 2021). Plant protein sources have been a major option to replace FM, but have raised several concerns, including food-feed competition, carbon footprint associated with land and water use and deforestation (Aubin et al., 2019; Hua et al., 2019). Moreover, the impact of plant-based feeds is highly dependent on the fish species considered (being more negative in carnivorous marine fish), and dietary inclusion level (Daniel, 2018; Naylor et al., 2021). In European seabass (*D. labrax*) juveniles, replacing FM by plant proteins (reducing FM from 59% to 16%), impaired immunity, with inhibition of immunoglobulins, and decreased monocytes percentage and gut interleukin 10 gene expression (Azeredo et al., 2017). According to Torrecillas et al. (2017), reducing FM from 20% to 5% (replacing it with soy protein concentrate, corn gluten and wheat gluten), for the same level of fish oil (6%), decreased seabass survival after a *Vibrio anguillarum* challenge. Likewise, Krogdahl and RØed (2000) and Siddik et al. (2021) showed that replacing FM with plant proteins in Atlantic salmon *Salmo salar* or barramundi *Lates calcarifer* diets decreased survival after infectious challenges, highlighting the need for a strategy to increase fish robustness when fed plant-protein rich diets.

Effective vaccination strategies are still lacking for many common bacterial infections in fish farms, and antibiotics tend to be the first choice to treat them (Gudding and Van Muiswinkel, 2013). However, antibiotic overuse can promote bacterial resistance, compromising the effectiveness of infection treatments and human health, as resistance may spread through the environment (Defoirdt et al., 2011; Santos and Ramos, 2018). Thus, the European Union has committed to reduce antibiotic use by 50% for all farmed animals by 2030 (European Commission, 2020). The dietary supplementation with bioactive compounds, and in particular, bioactive peptides, which are small chains of amino acids (AA) with benefits beyond their nutritional value, such as antioxidant, antimicrobial, immunostimulant, or others (Daroit and Brandelli, 2021), are good and sustainable options to boost fish production and enhance fish immunity. Dietary inclusion of marine hydrolysates, at levels below 5%, was shown to increase fish resistance to pathogens such as *Vibrio pelagius*, *Vibrio harveyi* and *Edwardsiella tarda* (Bui et al., 2014; Gisbert et al., 2018; Siddik et al., 2021). Hydrolysates are often obtained from protein rich coproducts, contributing to a circular economy framework (Faustino et al., 2019). Enzymatic hydrolysis is an increasingly used bioprocess, which when followed by filtration steps allows to fractionate the hydrolysate based on peptide size (Aiemratchanee et al., 2021; Ghalamara et al., 2020). Subcritical water extraction (or auto-hydrolysis) can also be a feasible alternative to enzymatic hydrolysis, since it is cheaper, less time-consuming, and could potentially result in higher peptide yields. Nevertheless, it is harder to control the peptide sequence or characteristics (Álvarez-Viñas et al., 2021; Lee et al., 2021).

Swine blood is a coproduct of meat production. Although there are no official reports of its global production, considering that nearly 246 million heads were produced in 2020 (European Commission, 2021) and that each slaughtered pork head results in 3.5 L of blood (Toldrà et al., 2019), it can be estimated that 859 million L of swine blood were collected in slaughterhouses over one year. This blood is further processed into low-cost blood meal (BM), and used as a protein source in animal feeds or for fertilizing, or eventually discarded (Hardy and Barrows, 2002). With BM price falling over the past years (Adhikari et al., 2018; Bah et al., 2013), its further processing into blood hydrolysates (BH) is a trend that allows value addition due to the generation of bioactive peptides and free amino acids (Bah et al., 2013).

The incorporation of BH in aquafeeds could improve feed intake, growth performance and mineral uptake, be immunomodulatory, enhance oxidative status or even boost fish response to stress or infectious events (Bah et al., 2013). However, when included in aquafeeds, the iron level, derived from hemoglobin, should be closely monitored to comply with the EU legal limits of 750 mg kg^{-1} of diet (Maage et al., 2008). Excess of iron can be toxic to fish, through decline of antioxidant defense system or respiratory dysfunction (Singh et al., 2019). BH from porcine blood included in microdiets, at a level of 9 or 12%, for gilthead seabream *Sparus aurata* larvae (Gisbert et al., 2012), was associated with lower malformities incidence and an earlier development of intestinal digestion (as evaluated by intestinal enzymes activity), when compared to a fish protein source. In juveniles, a plasma protein hydrolysate at a 5% inclusion level promoted fish growth and feed intake and enhanced the antibacterial activity of the skin mucus (Gisbert et al., 2021). The same hydrolysate included at 5% in diets for meagre (*Argyrosomus regius*) significantly improved skin mucus antibacterial activity, but reduced growth, compared to a non-supplemented FM-based control (Fernández-Alacid et al., 2021). Thus, the effects seem to be species dependent, and may also vary according to the hydrolysate type and inclusion level.

European seabass is a carnivorous fish, with a high protein requirement (up to 55% for fish below 20 g). As the industry is increasingly relying on plant-based feeds for this species, we've hypothesized that dietary inclusion of bioactive peptides, such as those in BH, in plant-based diets, could increase fish resistance to disease outbreaks, without compromising diet sustainability. Therefore, the aim of this study was to investigate the impact of swine BH inclusion, in plant-based diets for European seabass juveniles, on growth performance, nutrient utilization and resistance to an infectious challenge with *Tenacibaculum maritimum*, a bacterial species with high mortality in several farmed fish species.

2. Materials and methods

2.1. Ethical issues

Experiments were previously approved by the CIIMAR ethical committee for Managing Animal Welfare (ORBEA), in compliance with the Directive 2010/63/EU (European Union, 2010) and the Portuguese Decree Law n° 113/2013 on "The protection of animals used for scientific purposes". The present study was performed by accredited scientists in laboratory animal science by the Portuguese Veterinary Authority (1005/92, DGV-Portugal, following FELASA category C recommendations).

2.2. Blood hydrolysates (BH)

Swine blood was obtained at Central Carnes (Portugal) after bleeding the pigs with a sticking knife. Blood from animals considered safe for human consumption was collected in an adequate container, covered with a plastic bag, boiled at 100 °C for 45 min, and refrigerated until hydrolysis.

2.2.1. Enzymatic hydrolysis and filtration

Boiled swine blood (200 kg) was added to 200 L of water and the hydrolysis was performed using a *Cynara cardunculus* extract, with an enzyme: substrate ratio of 4% *v*/v, at a temperature of 55 °C and pH of 5.2, in an open vat under continuous mild agitation for 3 h. A first cloth filtration was applied, and the solids were discarded. Afterwards, the filtrate was subjected to microfiltration (MF). The process was performed in a batch equipment (Proquiga SA, Bergondo, Spain) using an organic MF membrane with 7.0 m^2 effective filtration area and a 500 kDa cut-off (PVDF/Polysulfone; FipoBiotech, Pontevedra, Spain). The retentate hydrolysate fraction from this microfiltration was called RMF-H. The filtrate was further fractionated, with a nanofiltration (3 kDa, 7.0 m^2 effective filtration area) membrane (PS/Polysulfone; FipoBiotech, Pontevedra, Spain). The retentate from this nanofiltration was named

RNF-H and it was concentrated through reverse osmosis, in a batch pilot plant (ORM, Belas, Portugal) equipped with a Polyamide Thin-Film Composite 2.6 m^2 membrane (2540FF, TW30LE-254D, Amfor ® Inc., USA). All this process is schematized in Fig. 1. The obtained hydrolysate fractions, RMF-H and RNF-H, were freeze-dried, prior to analysis and incorporation in diets. The peptide profile, proximate and mineral composition, and the AA profile of RMF-H and RNF-H are described in Table 1 and Fig. 2.

2.2.2. Autohydrolysis

Boiled swine blood was added to 800 mL of 0.1 M sodium hydroxide to a final solid load of 80 g L^{-1}. The hydrolysis was performed in a Parr 4523 (Parr®, Moline, USA) reactor, at a temperature of 160 °C, for 60 min, under agitation (160 rpm). The process was repeated until the desired final hydrolysate amount was achieved. The produced hydrolysate, named AH-H, was neutralized and oven-dried at 60 °C, prior to analysis and incorporation in diets. The peptide profile, proximate and mineral composition, and the AA profile of AH-H are described in Table 1 and Fig. 2.

2.3. Diet preparation

For the growth trial, five isonitrogeneous (54% protein in dry matter, DM) and isolipidic (16% DM) diets were formulated: a FM-based diet, including 25% of high quality FM, was the positive control (PC), and a practical plant-protein based diet, where 50% of that FM was replaced by vegetable proteins, was the negative control (NC); the three test diets (RMF, RNF, AH) were obtained by adding 3% of each BH to the NC at the expense of wheat gluten. All tested diets were formulated in conformity with European seabass nutrient requirements (National Research Council, 2011). Experimental diets were formulated and produced by SPAROS Lda. (Olhão, Portugal), through a pilot-scale twin-screw extruder (CLEXTRAL BC45, France) with a screw diameter of 55.5 mm at a temperature range of 105–110 °C. Mixtures, without oil, were oven-dried (OP 750-UF, LTE Scientifics, United Kingdom), for 3 h, at 60 °C, followed by oil supplementation under vacuum conditions in a Pegasus vacuum coater (PG-10VCLAB, DINNISEN, Netherlands) at room temperature. Experimental diets were stored at 4 °C until use. Ingredients and proximate composition of diets are described on Table 2.

2.4. Growth trial

The growth trial took place at CIIMAR facilities (Matosinhos, Portugal). Juvenile European seabass were obtained from a commercial fish farm (Acuinuga, S.L., Spain) and transported to the rearing facility. Fish were acclimatized for 15 days in a 2000 L tank, in a recirculating aquaculture system (RAS). Saltwater (35 ± 1‰) was kept at 20 ± 1 °C; nitrogen compounds and pH remained within the recommended ranges for this species, as described in Kır et al. (2019): ($NH_4^+ \leq 0.05$ mg L^{-1}; $NO_2^- \leq 0.5$ mg L^{-1}; $NO_3^- \leq 5$ mg L^{-1}; $7.5 \leq pH \geq 8.5$). Dissolved oxygen was always above 90% saturation, and the photoperiod was a cycle of 12 h light/12 h dark. Fish were hand-fed twice daily a commercial diet (AQUASOJA, Portugal; 50% crude protein and 20% crude fat as DM basis).

After 15 days of acclimation, fish were fasted for 24 h, slightly anesthetized (60 μL L^{-1} of 2-Phenoxyethanol; Sigma-Aldrich, MO, USA), individually weighed (12.3 ± 1.4 g), measured (total length; 10.8 ± 0.5 cm) and distributed into 15 homogeneous groups (71 fish/tank; density of 3.5 kg m^{-3}; CV =11%). Groups were kept in a RAS composed of 15 fiberglass tanks of 250 L. Each diet was randomly allocated to triplicate tanks. Each tank was provided with filtered, heated (20 ± 1 °C) saltwater (35 ± 1‰) at a flow rate of 16 L min^{-1}, under an artificial photoperiod of 12 h light/12 h dark. Physical and chemical water parameters (temperature, salinity, redox potential, pH and concentration of nitrogenous compounds) were regularly monitored during the trial and maintained at optimal levels for this species, as described above. Fish were hand-fed 3 times daily until apparent visual satiety, for a period of 74 days. Fish were bulk weighed in an intermediate sampling, after 5 weeks, to supervise feed consumption and weight gain. At the end of the growth trial, fish were fasted for 24 h before sampling. All fish were anesthetized (60 μL L^{-1} of 2-Phenoxyethanol) and individually weighed and measured for evaluation of growth performance, and feed consumption was registered. Additionally, 15 fish from the initial fish stock and 6 fish per tank at the end of the trial were collected, sacrificed with anesthetic overdose (0.5 mL L^{-1} of 2-Phenoxyethanol) and stored at −20 °C until chemical composition was analyzed. Blood was collected from the caudal vein of another 3 fish per tank (9 fish per treatment), using heparinized syringes, and centrifuged at 5000 g for 10 min at 4 °C. The obtained plasma was used for evaluation of immune parameters.

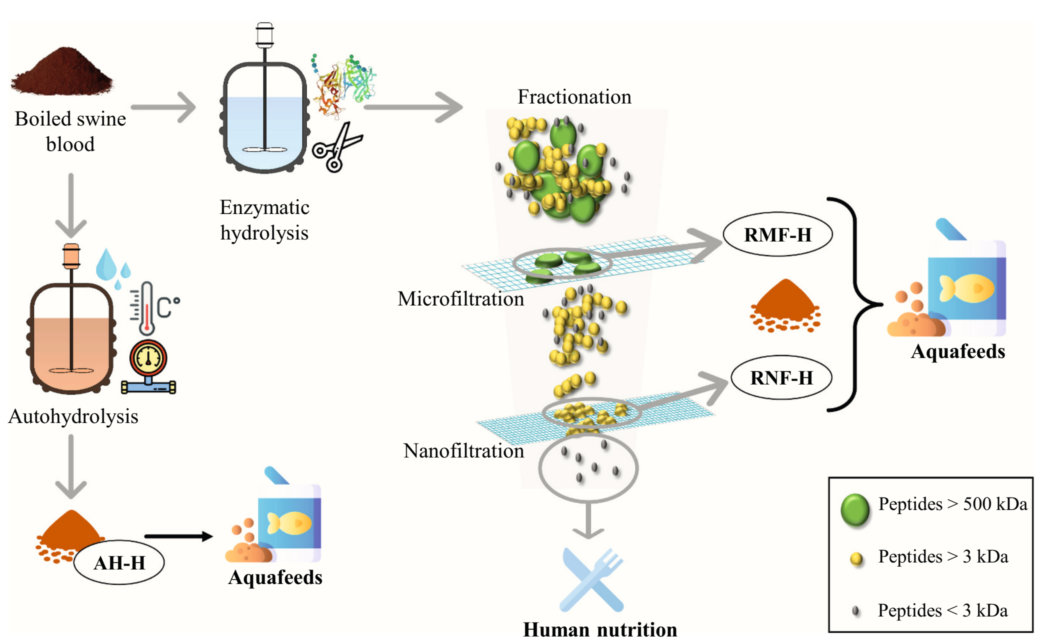

Fig. 1. Hydrolysate production schematized.

Table 1
Proximate composition, mineral composition and amino acid profile of the tested hydrolysates.

	Ingredients		
	RMF-H	RNF-H	AH-H
Peptide profile (mAU)			
>43 kDa	n.d.	n.d.	238,137
29–43 kDa	765	32,143	138,862
13.7–29 kDa	1513	14,786	80,132
1.2–13.7 kDa	48,017	373,302	349,651
<1.2 kDa	7656	79,596	131,995
Proximate composition (% DM)			
Dry matter (DM)	95.38	95.39	95.75
Ash	2.41	4.03	9.12
Crude protein	98.91	95.61	91.79
Crude fat	0.68	0.42	0.55
Energy (kJ/g DM)	23.76	22.11	22.01
Mineral composition (mg 100 g^{-1} DM)			
Zinc	1.31	n.d.	n.d.
Phosphorus	224.43	658.79	208.20
Iron	356.19	36.36	277.26
Magnesium	7.97	65.97	6.35
Calcium	n.d.	18.09	n.d.
Copper	0.79	0.55	0.72
Sodium	288.10	702.12	3103.11
Potassium	407.67	705.65	462.32
Amino acid profile (g 100 g^{-1} DM)			
Essential amino acids (EAA)			
Arginine	5.66	3.35	3.74
Histidine	8.46	5.68	7.07
Lysine	9.46	12.13	8.88
Threonine	3.33	3.35	1.58
Isoleucine	1.03	0.33	1.16
Leucine	11.68	9.74	11.62
Valine	7.95	7.44	8.05
Methionine	0.71	1.67	0.04
Phenylalanine	6.92	6.33	6.59
$\sum$ EAA	55.20	50.01	48.72
Non-essential amino acids			
Cystine	0.49	0.09	0.21
Tyrosine	2.93	2.03	2.67
Aspartic acid + asparagine	9.89	9.49	10.45
Glutamic acid + glutamine	8.03	8.67	8.75
Alanine	6.80	9.74	7.24
Glycine	5.48	8.30	7.19
Proline	3.82	2.46	3.91
Serine	5.18	5.39	3.00
$\sum$ NEAA	42.62	46.17	43.43
$\sum$ AA	97.82	96.17	92.15

DM – dry matter. n.d. not detectable (<0.1 mg 100 g^{-1}).

These fish were then sacrificed by a sharp blow on the head and viscera and liver were weighed to calculate viscerosomatic (VSI) and hepatosomatic (HSI) indices, respectively. An anterior intestine cross-section (0.5 cm) was also sampled, washed and fixed in 10% neutral-buffered formalin for 24 h, followed by preservation in ethanol 70% until further histological processing.

2.5. Infectious challenge

After the growth trial, 10 fish from each tank were transferred to a new rearing system with 130 L tanks with strong aeration for inoculation with *Tenacibaculum maritimum* (strain ACC13.1) during 2 h. This bacterial species was isolated from Senegalese sole in a local fish farm (Portugal) and kindly provided by Prof. Alicia Toranzo (University of Santiago de Compostela, Spain). Bacteria were cultured at 22 °C in marine agar for 48 h and then inoculated in marine broth for 18 h. To prepare the inoculum, exponentially growing bacteria in marine broth (MB) were collected and adjusted to a final concentration of 3.5×10^5 colony forming units (cfu) mL^{-1}, which was used for inoculation. This dosage was selected after a pre-challenge to determine 30% lethal dose (LD30). The recirculation water system was stopped and the water volume of each tank was lowered to a final volume of 10 L. Then, fish were inoculated with the bacteria in a bath challenge with strong aeration for 2 h. After the 2 h infectious challenge, the water of each tank was changed three times and the recirculation was re-established, under the same experimental conditions as the growth trial. Fish were daily supervised and fed the same diets as the growth trial. Dead and moribund fish were collected/euthanized and examined for the detection of *T. maritimum* by bacteria recovery in head-kidney in MB, and the cumulative mortality of each group was assessed until the 8th day after infection considering the formula:

Mortality = number of dead animals / number of total animals × 100.

Any survivors at the end of this challenge trial were euthanized by an anesthetic overdose (1 mL L^{-1} 2-phenoxyethanol).

2.6. Digestibility trial

For the digestibility trial, the same diets as described in section 2.3. were used, with the addition of yttrium oxide (200 mg kg^{-1} of diet) as an inert marker. After the growth trial, 10 groups of 15 fish per tank were transferred to a RAS composed of 10 fiberglass tanks of 50 L with individual feces sedimentation columns, Guelph system (Cho and Slinger, 1979) and 5 groups (one of each dietary treatment) were transferred to similar tanks but without sedimentation columns (for acclimation until a second run was performed). Water parameters and photoperiod remained as described for the growth trial. Fish were acclimatized to the new system for two weeks, keeping the same diets they were fed during the growth trial. After this period, fish were hand-fed twice daily (9:00 and 17:00 h) with the marked diets, until apparent satiation. After feeding, the tanks were thoroughly cleaned until no residues of feed were left. Feces were always collected prior to feeding and centrifuged at 3000 *g* to eliminate water excess before freezing at −20 °C. Feces were collected for two weeks. After this period, a second run was carried out with the remaining five groups of fish, one from each dietary treatment, to obtain triplicates for each dietary treatment. Prior to analysis, feces were freeze-dried, ground and sifted.

2.7. Chemical analysis

2.7.1. Proximate analysis

Hydrolysates, diets and feces were ground prior chemical analysis that were performed in duplicate and according to AOAC methods. Dry matter (DM) was evaluated after 24 h at 105 ± 1 °C; ash was determined by combustion in a muffle furnace (Nabertherm L9/11/B170; Bremen, Germany; 550 °C for 6 h); crude protein (CP; N × 6.25) was measured using a Leco nitrogen analyzer (Model FP-528, Leco Corporation, St. Joseph, USA); crude fat (CF) by petroleum ether (40–60 °C) extraction (CF, SoxtecTM 2055, Foss, Höganäs, Sweden) and gross energy in an adiabatic bomb calorimeter (Werke C2000, IKA, Staufen, Germany) calibrated with benzoic acid.

For the determination of whole-body composition, fish from each tank were ground and pooled and moisture was calculated (105 °C for 24 h). Afterwards, fish were freeze-dried for further analysis of DM, ash, CP and CF, following the methods described above. Additionally, phosphorus content was determined from ashes by digestion at 150 °C in hydrochloric acid, followed by the quantification of phosphates using ammonium molybdate (2.5 mg mL^{-1}) at 75 °C in a water bath and later determination of absorbance at 820 nm (ISO 13730, 1996).

2.7.2. Mineral analysis

Mineral analysis (mineral profiling and quantification of the inert marker, yttrium, on diets and feces) of hydrolysates, diets and feces was performed as described by Castro et al. (2022), with some modifications. Briefly, 300 mg of each sample were digested with 9 mL nitric acid (65%, Merck KGaA, Darmstadt, Germany) and 1 mL hydrogen peroxide (30%, Merck KGaA, Darmstadt, Germany), using a microwave digester

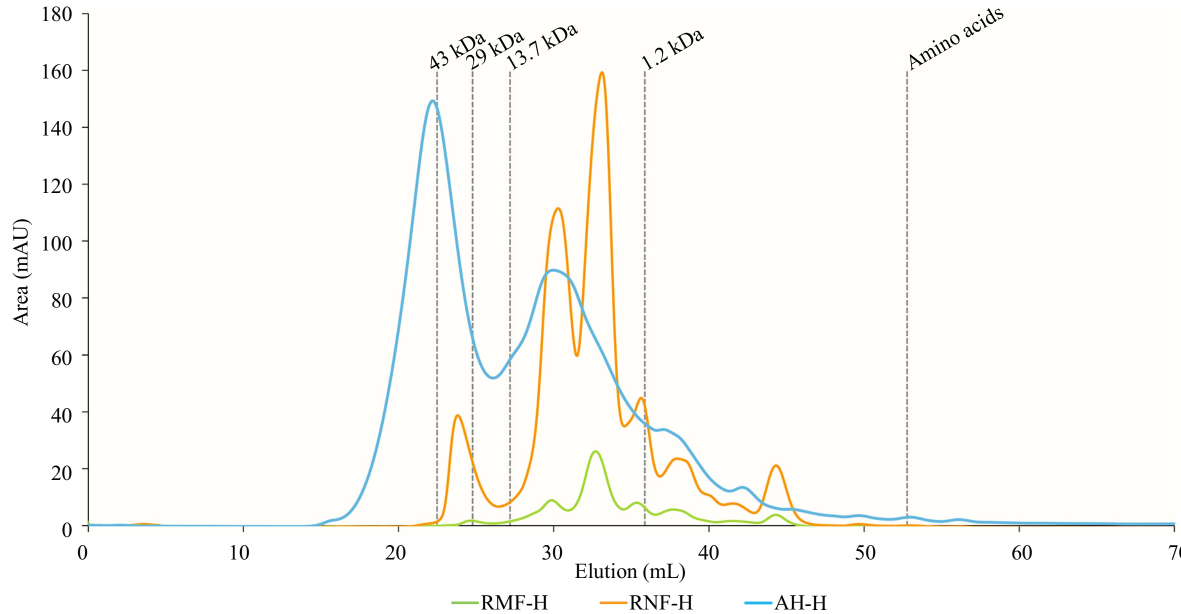

Fig. 2. Peptide profile by size of molecular weight of solubilised RMF-H, RNF-H and AH-H. Adapted from Araújo-Rodrigues et al. (2022).

(Berghof, Germany). The digestion program used was as follows: step (1) 30 min to reach 130 °C (5% power), step (2) 40 min to reach 170 °C (10% power), step (3) 50 min to reach 200 °C (15% power), step (4) 30 min to reach 100 °C (2% power), and step (5) 20 min to reach 100 °C (2% power). Pressure was maintained at 20 bar. Digestion bottles were previously washed to prevent cross-contamination. Afterwards, digested samples were cooled in an ice bath, collected into 50 mL polyethylene bottles, made up to 20 mL with ultrapure water and kept at 4 °C until analysis. Mineral content was assessed by inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin Elmer, Optical Emission Spectrometer, Optima 7000 DV, Waltham, USA). External calibration curves of Zn (zinc; 0.06–6 mg L^{-1}; 213.857 nm), P (phosphorus; 2.5–250 mg L^{-1}; 214.914 nm), Mn (manganese; 0.025–2.5 mg L^{-1}; 257.610 nm), Fe (iron; 0.15–15 mg L^{-1}; 259.939 nm), Mg (magnesium; 1.5–150 mg L^{-1}; 279.077 nm), Ca (calcium; 5–500 mg L^{-1}; 317.933 nm), Cu (copper; 0.015–1.5 mg L^{-1}; 324.752 nm), Al (aluminium; 0.025–2.5 mg L^{-1}; 394.401 nm), Na (sodium; 0.25–25 mg L^{-1}; 589.592 nm), K (potassium; 6–600 mg L^{-1}; 769.896 nm) and Y (yttrium; 0.02–10 mg L^{-1}; 371.029 nm) were prepared in nitric acid 5% from a standard mix (UCP-3 from Inorganic Ventures) and a yttrium standard (E1196, Fluka). Mineral concentrations were calculated based on the calibration curves and values were expressed as mg element 100 g^{-1} diet.

2.7.3. AA analysis

AA analysis was performed as described in Teodósio et al. (2021). Briefly, samples from the tested ingredients were hydrolyzed (6 M chloridric acid, at 116 °C, for 72 h in nitrogen-flushed glass vials), prior to total AA analysis. Afterwards, samples were pre-column derivatized with Waters AccQ Fluor Reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) using the AccQ Tag method (Waters, USA). Ultra-high-performance liquid chromatography (UPLC) was employed for analysis, in a Waters Reversed-Phase Amino Acid Analysis System, using norvaline as an internal standard. Considering tryptophan is partially lost during the acid hydrolysis, its content was not measured. The obtained peaks were evaluated with EMPOWER software (Waters, USA).

2.7.4. Peptide profile analysis

The peptide profile analysis was conducted as described in Voss et al. (2019) and Araújo-Rodrigues et al. (2022). Molecular weight of BH was evaluated by size exclusion chromatography with the AKTA Pure 25 system (GE Healthcare Life Sciences, Freiburg, Germany).

2.8. Anterior intestine histomorphology

Anterior intestine samples were embedded in paraffin and then processed according to standard histological procedures. One cross-section from the anterior intestine (3 μm) of each fish, obtained with a semi-automated rotary microtome (Leica RM 2245, Nußloch, Germany), was either stained with haematoxylin and eosin (H&E) for qualitative structural analysis of the intestinal morphology, or Alcian blue/periodic acid Schiff (AB-PAS; pH 2.5) for intestinal quantitative measurements. H&*E*-stained sections were analyzed under a light microscope (Olympus BX51, GmbH, Hamburg, Germany) and the morphology was evaluated considering: width and cellularity of submucosa and *lamina propria*; mucosal folds' morphology; presence of inflammatory infiltrate; vacuolization and nucleus position of the enterocytes. Micrographs of AB-PAS sections were taken, and an imaging software (Olympus cellSens Dimension Desktop, Tokyo, Japan) was used to assess the following parameters: cross-sectional perimeter; width of *muscularis externa*, submucosa and *lamina propria* (eight measurements per section); total number of goblet cells (both acid and neutral cells were counted in each section); and *villus* length (the eight longest *villi* were measured), as represented in Fig. 3C, and according to Batista et al. (2020).

2.9. Humoral immune parameters

Lysozyme, peroxidase and alternative complement pathway activity (ACH50) activities were determined as described in Costa et al. (2020), with triplicate measurements in a microplate spectrophotometer (BioTekSynergy HT, VT, USA). Plasma lysozyme activity was determined using a turbidimetric assay adapted to microtiter, using a calibration curve with serially diluted, lyophilized hen egg white lysozyme (Sigma). Plasma peroxidase activity (EU mL^{-1}) was measured considering one peroxidase EU produced an absorbance change of 1 in the optical density. ACH50 was based on the lysis of rabbit red blood cells (2.8×10^8 cells mL^{-1}), and ACH50 units were set as the concentration of plasma giving 50% cell lysis.

Table 2
Formulation, proximate composition and mineral composition of the diets used in the growth trial.

	Diets				
	PC	NC	RMF	RNF	AH
Ingredients (%)					
Fishmeal [a]	25.00	12.50	12.50	12.50	12.50
Soy protein concentrate [b]	25.00	25.00	25.00	25.00	25.00
Wheat gluten [c]	10.70	13.50	10.00	10.10	10.20
Corn gluten [d]	7.50	15.00	15.00	15.00	15.00
Soybean meal 48 [e]	5.00	10.00	10.00	10.00	10.00
Wheat meal [f]	12.10	7.24	7.44	7.34	7.34
Fish oil [g]	13.00	13.40	13.70	13.70	13.60
Vit & Min Premix [h]	0.50	0.50	0.50	0.50	0.50
DCP [i]	1.20	2.80	2.80	2.80	2.80
L-Tryptophan	0	0.06	0.06	0.06	0.06
RMF-H [j]	0	0	3.00	0	0
RNF-H [k]	0	0	0	3.00	0
AH-H [l]	0	0	0	0	3.00
Proximate composition (%DM)					
Dry matter (DM, %)	96.92	96.24	94.63	97.00	93.96
Ash	8.49	7.93	7.73	7.87	8.04
Crude protein	54.42	54.24	54.72	54.57	54.63
Crude fat	15.81	15.93	15.79	16.21	16.03
Energy (kJ/g DM)	22.14	22.37	22.44	22.55	22.69
Mineral Composition (mg 100 g^{-1} DM)					
Zinc	3.36	3.45	3.73	3.54	27.29
Phosphorus	981.67	1083.15	987.24	1048.70	1019.31
Manganese	2.67	2.89	2.46	3.13	9.97
Iron	9.21	9.84	17.92	10.42	53.72
Magnesium	157.25	143.92	142.62	144.44	133.51
Calcium	1714.62	1732.17	1473.87	1622.79	1241.85
Copper	1.78	1.76	2.71	1.67	3.21
Aluminium	6.51	6.59	6.13	6.49	5.52
Sodium	427.64	248.05	238.30	290.63	307.08
Potassium	976.69	941.22	992.17	958.63	929.38

[a] Peruvian fishmeal (71% crude protein, 11% crude fat), EXALMAR, Peru.
[b] Soy protein concentrate (65% protein, 0,7% lipids), ADM, Animal Nutrition ™, The Netherlands.
[c] Wheat gluten composition: DM: 901 g kg^{-1}; protein: 838 g kg^{-1} DM; lipids: 16 g kg^{-1}.
[d] Corn gluten feed: 61% CP, 6% CF, COPAM, Portugal.
[e] Dehulled solvent extracted soybean meal (47.7% CP, 2.2% CF), CARGILL, Spain.
[f] Wheat meal (10.2% protein, 1.2% lipids), Casa Lanchinha Lda., Portugal.
[g] Sardine oil, Sopropêche, France.
[h] Vitamin and mineral premix: INVIVO 1%, Premix for marine fish, PREMIX Lda, Portugal. Vitamins (IU or mg kg^{-1} diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20,000 IU; DL-cholecalciferol, 2000 IU; thiamine, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 1000 mg; inositol, 500 mg; biotin, 3 mg; calcium pantothenate, 100 mg; choline chloride, 1000 mg, betaine, 500 mg. Minerals (g or mg kg^{-1} diet): cobalt carbonate, 0.65 mg; copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate,7.5 mg; sodium chloride, 400 mg; calcium carbonate, 1.86 g; excipient wheat middlings.
[i] Di-calcium phosphate.
[j] Blood hydrolysate: retentate from microfiltration.
[k] Blood hydrolysate: retentate from nanofiltration.
[l] Blood hydrolysate: produced by auto-hydrolysis.

2.10. Calculations

The calculations for the assessment of growth performance, feed efficiency and digestibility were as follows:

Apparent digestibility coefficient of DM (ADC DM, %) = 100 × [1 – (dietary Y level / feces Y level)].

Nutrient ADC (%) = 100 × [1 – (dietary Y level / feces Y level) × (feces nutrient level / dietary nutrient level)].

Crude nutrient intake (g $kg^{-1}d^{-1}$) = dry nutrient intake, g / (ABW × trial duration, d).

Digestible nutrient intake (g kg^{-1} d^{-1}) = crude nutrient intake (g kg^{-1} d^{-1}) × Nutrient ADC.

Nutrient gain (g kg^{-1} d^{-1}) = (final carcass nutrient content, g – initial carcass nutrient content, g) / (ABW × trial duration, d) × 100.

Nutrient retention efficiency (% digestible intake) = (Nutrient gain /Digestible Nutrient intake) × 100.

Fecal nutrient losses = Crude nutrient intake × [1 – (ADC nutrient/ 100)].

Non-fecal nutrient losses = Digestible nutrient intake – nutrient gain.

Metabolizable energy = digestible energy intake – non-fecal energy loss.

Total heat loss = Metabolizable energy – Energy gain.

Total energy loss = Energy intake – Energy gain.

Condition index (K) = 100 × final weight, g / (final 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.

Average body weight (ABW, kg) = (initial body weight, kg + final body weight, kg) / 2.

Hepatosomatic index (HSI) = liver weight, g / body weight, g × 100.

Viscerosomatic index (VSI) = viscera weight, g / body weight, g × 100.

2.11. Statistical analysis

Results are expressed as means ± standard deviation. All data were tested for normality and homogeneity of variances by Kolmogorov-Smirnov and Levene's tests, respectively, and adequately transformed if required. One-way ANOVA was applied to analyze data, using the SPSS (IBM SPSS Statistics 26, IL, USA) software. Whenever significant effects of treatments were detected, means were compared through the pairwise Tukey multiple comparison test. If data did not meet the assumptions of ANOVA, a Kruskal-Wallis test was carried out for each factor and the pairwise multiple comparison of mean ranks was performed to detect significant differences between groups. Regarding the infectious challenge, the Chi-square test was performed to identify differences on the cumulative mortality among dietary treatments. The minimum level of significance was set at $p < 0.05$ for all analysis. Data were further analyzed using Principal Component Analysis (PCA) with the Statistica (v 13.5.0.17) software (TIBCO Software, CA, USA).

3. Results

3.1. Hydrolysates and diets characterization

RMF-H displayed the lowest total area (Fig. 2; Table 1) in the fast protein liquid chromatography (FPLC) analysis associated to its lower protein solubility. RNF-H showed the highest area in the fraction of 1.2–13.7 kDa (Table 1), but also has a visible peak below 1.2 kDa, (with an elution volume near 45 mL), which was not observed in the other BH (Fig. 2; adapted from Araújo-Rodrigues et al., 2022). All tested BH had a high crude protein content (> 90%, dry matter basis), and a very low crude fat content (< 1%; Table 1). Ash content varied between 2 and 9% and was highest in AH-H, followed by RNF-H and RMF-H. This was reflected in the mineral composition. AH-H had an extremely high level of sodium compared to all other BHs (31 vs 3–7 mg/g) and was a rich source of potassium. However, RNF-H had the highest levels of potassium, also being a rich source of phosphorus, magnesium and sodium, whilst RMF-H was particularly rich in iron and potassium.

Concerning the AA profile of the hydrolysates, essential AA

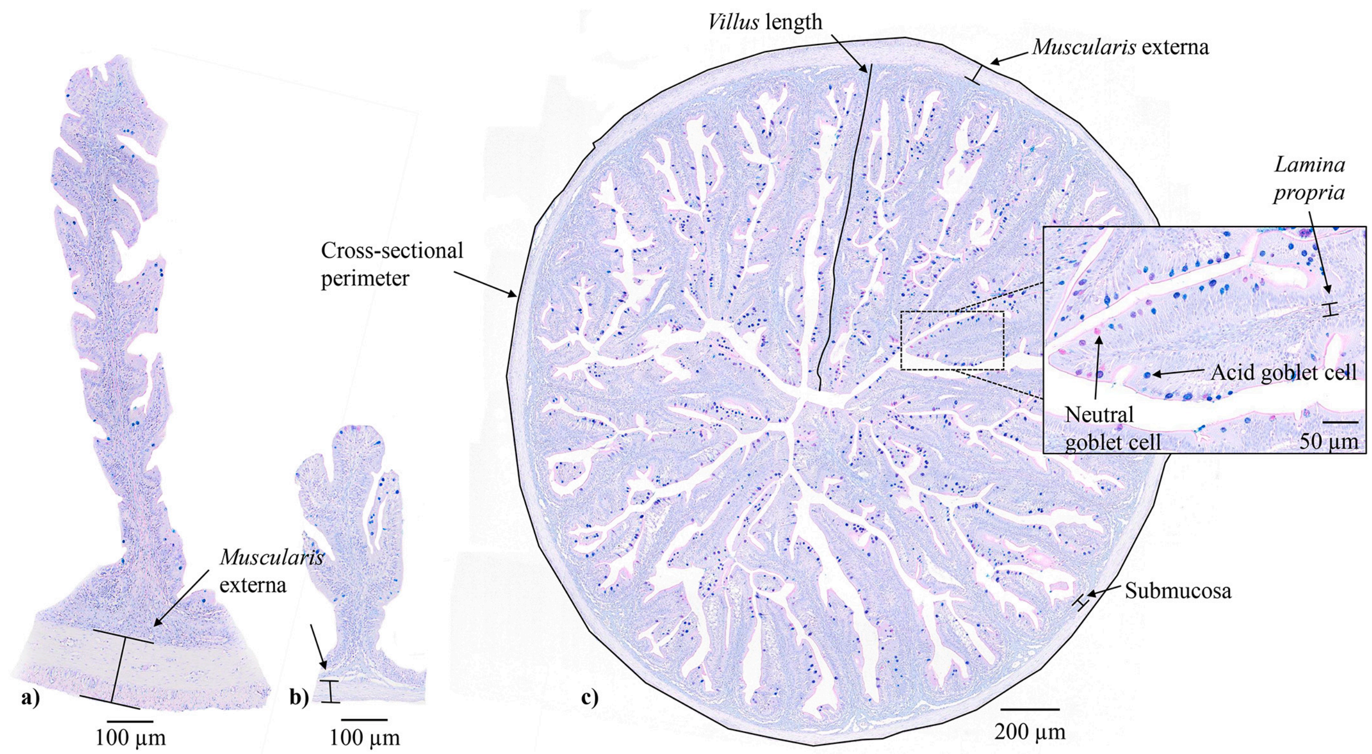

Fig. 3. Anterior intestine histological cross-sections (AB/PAS staining) of European seabass fed the experimental diets. Note the reduced villus length and muscularis externa in fish fed RMF (b) compared to those fed the NC (a). All performed measurements are represented in the whole cross-section (c).

contributed with 49–55%, whilst non-essential AA with 43–46% to total amino acids. Lysine and leucine were the most abundant essential AA, followed by histidine and valine. Aspartic acid/asparagine, glutamic acid/glutamine and alanine were the most abundant non-essential AA in all fractions.

Regarding the experimental diets, the most abundant minerals were calcium, phosphorus, potassium and magnesium. Iron levels were highest for AH diet (53.7 mg 100 g^{-1}), followed by RMF, RNF, NC and finally PC (Table 2).

3.2. Apparent digestibility of the experimental diets and nutrient balance

The apparent digestibility coefficients (ADC) varied significantly among experimental diets (Table 3). Dry matter ADC was significantly lower in hydrolysates-containing diets compared to the PC; RNF did not differ from the NC. Protein ADC was generally high (>91%) and both RMF and RNF had similar values to the PC and NC diets. AH had the lowest protein ADC value. RMF and AH had significantly lower lipid ADCs when compared to PC and NC values, while lipid ADC of RNF did not differ from that of NC. Energy digestibility was highest for PC and NC diets, followed by RNF and RMF, being lowest in AH.

Mineral ADC values of the RNF did not differ statistically from neither the NC nor the PC. Phosphorus and manganese digestibility were unaffected by the treatments. AH diet had a significantly lower iron ADC value compared to both controls. Calcium ADC was significantly lower in RMF and AH diets than in both the NC and PC diets. RMF diet displayed the lowest copper and potassium ADC value.

Digestible nitrogen intake was highest in fish fed PC, although this was only significantly higher when compared to the RMF diet. No significant differences were found on the nitrogen gain, nitrogen retention efficiency and non-fecal nitrogen losses among dietary treatments. Digestible lipid intake was lowest for RMF and differed significantly from both PC and NC. Lipid gain and retention efficiency were lowest for RMF, and they were significantly lower than all the remaining diets, which did not differ among themselves. Lipid fecal losses were higher for hydrolysate-containing diets when compared to both controls, and they followed the order: AH > RMF > RNF. Digestible phosphorus intake was maximum for the RNF diet, which did not differ from the NC diet. Phosphorus gain, retention efficiency and non-fecal losses were not significantly affected by dietary treatments. Phosphorus fecal losses were highest for RMF, followed by AH, the controls and finally RNF. The digestible energy intake and the metabolizable energy was highest for controls, followed by RNF, AH and RMF. Energy gain and retention efficiency were lowest for RMF and similar among the remaining diets. Fecal energy losses were significantly higher in hydrolysate-containing diets, in the following order: AH > RMF > RNF. Total heat losses were highest in fish fed RMF, albeit statistically similar to AH. RNF did not differ from either controls in this parameter.

3.3. Growth performance and whole-body composition

Fish fed PC had the highest final weight, followed by NC and RNF diets that had similar final weights. The lowest final body weight was found in RMF-fed fish. The same trend was observed for final body length. Mortality was very low; it was null for PC and RNF diets, and < 1.5% for the remaining treatments, without differences among diets. Daily growth index (DGI) and feed conversion ratio (FCR) of fish fed RNF showed no significant differences from those fed PC and NC. Diet RMF showed the highest FCR and lowest DGI. No differences were found in PER values for PC and RNF. The voluntary feed intake (VFI) was not significantly affected by dietary treatments.

No significant differences were found for HSI among dietary groups, but VSI was significantly lower in fish fed RMF compared to those fed all remaining diets. Final whole-body composition was not significantly affected by the different diets (Table 4).

Table 3
Apparent digestibility coefficients of macronutrients and minerals of the tested diets.

	Diets					*P* value
	PC	NC	RMF	RNF	AH	
ADC (%)						
Dry matter	76.99 ± 1.78^{a}	74.80 ± 1.36^{ab}	60.96 ± 5.35^{c}	66.87 ± 1.51^{bc}	60.94 ± 4.86^{c}	<0.001
Protein	95.54 ± 0.13^{a}	95.20 ± 0.20^{ab}	91.55 ± 1.29^{ab}	92.84 ± 0.55^{ab}	91.37 ± 0.64^{b}	0.017
Lipid	97.95 ± 0.17^{a}	97.68 ± 0.17^{ab}	92.46 ± 2.12^{c}	94.41 ± 1.22^{bc}	91.30 ± 1.52^{c}	<0.001
Energy	90.19 ± 0.48^{a}	89.47 ± 0.64^{a}	77.14 ± 3.60^{bc}	81.59 ± 0.89^{b}	76.73 ± 0.70^{c}	<0.001
Phosphorus	60.27 ± 4.93	61.15 ± 8.20	51.01 ± 2.96	63.60 ± 2.28	58.16 ± 2.92	0.071
Manganese	58.52 ± 10.55	66.37 ± 7.39	65.70 ± 9.03	72.55 ± 3.28	70.36 ± 5.98	0.272
Iron	16.90 ± 1.90^{a}	15.82 ± 4.79^{a}	12.33 ± 0.87^{ab}	8.22 ± 1.44^{ab}	6.28 ± 3.18^{b}	0.015
Calcium	60.17 ± 5.52^{a}	59.15 ± 8.79^{a}	39.46 ± 2.20^{b}	59.22 ± 3.67^{a}	38.50 ± 2.08^{b}	0.001
Copper	80.89 ± 2.08^{ab}	85.74 ± 1.01^{a}	51.90 ± 4.56^{b}	80.28 ± 0.38^{ab}	84.66 ± 1.30^{ab}	0.015
Potassium	86.68 ± 1.32^{a}	84.26 ± 1.22^{ab}	72.53 ± 5.46^{b}	80.63 ± 2.03^{ab}	76.59 ± 5.59^{ab}	0.017
Nitrogen (N) balance (mg 100 g ABW $^{-1}$ day $^{-1}$)						
DNI	162.59 ± 4.65^{a}	156.73 ± 0.50^{ab}	152.03 ± 1.52^{b}	156.67 ± 3.38^{ab}	157.78 ± 2.37^{ab}	0.016
N gain	46.22 ± 2.00	46.29 ± 1.37	38.73 ± 5.12	44.00 ± 0.83	45.04 ± 1.59	0.106
NRE (% DN)	28.44 ± 1.33	29.53 ± 0.86	25.46 ± 3.11	28.10 ± 0.78	28.54 ± 0.65	0.246
Fecal N losses	7.59 ± 0.22^{d}	7.91 ± 0.03^{d}	14.03 ± 0.14^{b}	12.08 ± 0.26^{c}	14.90 ± 0.22^{a}	<0.001
Non-fecal N losses	116.37 ± 4.69	110.44 ± 1.36	113.30 ± 3.70	112.66 ± 3.42	112.74 ± 1.18	0.321
Lipid (L) balance (g kg ABW $^{-1}$ day $^{-1}$)						
DLI	3.03 ± 0.09^{a}	2.95 ± 0.01^{a}	2.77 ± 0.03^{b}	2.96 ± 0.06^{a}	2.89 ± 0.04^{ab}	0.002
L gain	3.10 ± 0.25^{a}	2.99 ± 0.16^{a}	1.59 ± 0.49^{b}	2.83 ± 0.16^{a}	2.83 ± 0.19^{a}	<0.001
LRE (% DL)	102.28 ± 7.92^{a}	101.24 ± 5.69^{a}	57.42 ± 17.31^{b}	95.83 ± 6.73^{a}	97.93 ± 5.80^{a}	0.001
Fecal L losses	0.06 ± 0.01^{d}	0.07 ± 0.01^{d}	0.23 ± 0.01^{b}	0.18 ± 0.01^{c}	0.28 ± 0.01^{a}	<0.001
Phosphorus (P) balance (mg 100 g ABW $^{-1}$ day $^{-1}$)						
DPI	12.37 ± 0.35^{c}	12.99 ± 0.04^{ab}	10.55 ± 0.11^{d}	13.27 ± 0.29^{a}	12.41 ± 0.19^{bc}	<0.001
P gain	9.27 ± 2.25	9.18 ± 1.29	10.00 ± 0.10	8.45 ± 2.11	8.87 ± 1.76	0.843
PRE (% DP)	75.16 ± 19.02	70.65 ± 9.78	94.81 ± 0.15	63.76 ± 16.39	71.62 ± 15.27	0.149
Fecal P losses	8.15 ± 0.23^{c}	8.25 ± 0.03^{c}	10.13 ± 0.10^{a}	7.60 ± 0.16^{d}	8.93 ± 0.13^{b}	<0.001
Non-fecal P losses	3.09 ± 2.41	3.81 ± 1.27	0.55 ± 0.02	4.83 ± 2.25	3.54 ± 1.95	0.125
Energy (E) balance (kJ kg ABW $^{-1}$ day $^{-1}$)						
DEI	390.29 ± 11.16^{a}	379.69 ± 1.20^{a}	328.32 ± 3.29^{c}	355.59 ± 7.67^{b}	343.96 ± 5.17^{bc}	<0.001
E gain	174.47 ± 8.02^{a}	174.00 ± 3.82^{a}	114.18 ± 23.67^{b}	163.29 ± 7.75^{a}	167.35 ± 6.41^{a}	0.001
ERE (%DE)	44.70 ± 1.52^{a}	45.83 ± 1.08^{a}	34.73 ± 6.86^{b}	45.95 ± 2.81^{a}	48.65 ± 1.49^{a}	0.006
Fecal E losses	42.43 ± 1.21^{d}	44.68 ± 0.14^{d}	97.29 ± 0.97^{b}	80.23 ± 1.73^{c}	104.30 ± 1.57^{a}	<0.001
Non-fecal E losses	28.98 ± 1.17	27.50 ± 0.34	28.21 ± 0.92	28.05 ± 0.85	28.07 ± 0.29	0.321
Metabolizable E	361.31 ± 10.11^{a}	352.19 ± 1.19^{a}	300.11 ± 4.15^{c}	327.54 ± 6.85^{b}	315.89 ± 4.93^{bc}	<0.001
Total energy loss	258.25 ± 9.01^{b}	250.37 ± 4.58^{b}	311.44 ± 19.45^{a}	272.53 ± 14.91^{b}	280.92 ± 5.35^{ab}	0.001
Total heat loss	186.84 ± 7.04^{a}	178.19 ± 4.20^{ab}	185.94 ± 19.64^{a}	164.24 ± 12.79^{ab}	148.54 ± 4.98^{b}	0.009

ADC – apparent digestibility coefficients; ABW – average body weight; DNI – digestible nitrogen intake; NRE – nitrogen retention efficiency; DLI – digestible lipid intake; LRE – lipid retention efficiency; DPI – digestible phosphorus intake; PRE – phosphorus retention efficiency; DEI – digestible energy intake; ERE – energy retention efficiency.
Values represent mean ± standard deviation. In each row, different letters indicate significant differences between treatments ($p < 0.05$).

Table 4
Growth performance indicators and final whole-body composition of European seabass fed the experimental diets.

	Diets					*P* value
	PC	NC	RMF	RNF	AH	
Final BW (g)	51.73 ± 8.68^{a}	48.93 ± 8.21^{b}	33.37 ± 5.27^{d}	47.01 ± 7.30^{bc}	46.62 ± 7.76^{c}	<0.001
Final BL (cm)	16.3 ± 0.8^{a}	16.1 ± 0.9^{b}	14.6 ± 0.8^{d}	15.9 ± 0.8^{bc}	15.9 ± 0.9^{c}	<0.001
K	1.18 ± 0.03^{a}	1.17 ± 0.02^{a}	1.07 ± 0.01^{b}	1.16 ± 0.01^{a}	1.16 ± 0.02^{a}	<0.001
DGI	1.91 ± 0.08^{a}	1.82 ± 0.06^{ab}	1.23 ± 0.03^{c}	1.75 ± 0.07^{ab}	1.74 ± 0.06^{b}	<0.001
FCR	1.18 ± 0.04^{c}	1.17 ± 0.02^{c}	1.52 ± 0.01^{a}	1.22 ± 0.02^{bc}	1.26 ± 0.02^{b}	<0.001
PER	1.56 ± 0.05^{ab}	1.57 ± 0.03^{a}	1.20 ± 0.01^{d}	1.50 ± 0.02^{bc}	1.46 ± 0.02^{c}	<0.001
VFI (g kg ABW^{-1} day^{-1})	19.54 ± 0.56	18.97 ± 0.06	18.97 ± 0.19	19.33 ± 0.42	19.76 ± 0.30	0.074
HSI	1.64 ± 0.20	1.78 ± 0.26	1.76 ± 0.30	1.74 ± 0.18	1.75 ± 0.24	0.133
VSI	8.31 ± 1.75^{a}	8.32 ± 1.95^{a}	6.52 ± 1.21^{b}	8.70 ± 1.53^{a}	8.71 ± 1.59^{a}	<0.001
WBC (%WW)						
Dry matter	36.63 ± 1.48	36.81 ± 1.09	33.59 ± 3.93	35.87 ± 1.09	36.50 ± 0.58	0.780
Ash	3.75 ± 0.52	3.67 ± 0.22	4.62 ± 0.39	3.57 ± 0.54	3.68 ± 0.54	0.096
Protein	17.19 ± 0.29	17.58 ± 0.51	18.39 ± 1.52	17.20 ± 0.45	17.56 ± 0.41	0.420
Lipid	15.98 ± 0.92	15.75 ± 0.89	10.85 ± 2.44	15.21 ± 0.76	15.24 ± 0.72	0.102
Energy (kJ/g)	9.62 ± 0.22	9.78 ± 0.28	8.30 ± 1.14	9.42 ± 0.37	9.63 ± 0.17	0.187
Phosphorus	0.63 ± 0.10	0.64 ± 0.05	0.82 ± 0.01	0.61 ± 0.10	0.64 ± 0.09	0.045*

BW – body weight; BL – body length; K – condition factor; DGI – daily growth index; FCR – feed conversion ratio; PER – protein efficiency ratio; VFI – voluntary feed intake; ABW – average body weight; HSI – hepatosomatic index; VSI – viscerosomatic index; WBC – Whole-body composition; WW – Wet weight.
Values represent mean ± standard deviation ($n = 3$ except for BW and BL where $n = 213$). In each row, different letters indicate significant differences between treatments ($p < 0.05$). Initial whole-body composition (% or kJ/g wet weight): DM, 29.55; ash, 4.85; protein, 16.57; lipids, 7.54; energy, 6.81; phosphorus, 0.84. In each row, different letters indicate significant differences between treatments ($p < 0.05$). * Without significant differences after post-hoc analysis.

Table 5
Anterior intestine morphology of European seabass fed the experimental diets.

	Diets					*P* value
	PC	NC	RMF	RNF	AH	
Cross sectional perimeter (mm)	10.8 ± 1.2 [a]	11.0 ± 1.5 [a]	8.7 ± 1.4 [b]	11.3 ± 2.2 [a]	10.9 ± 1.4 [a]	0.008
Muscularis externa (μm)	97.3 ± 32.3 [a]	106.6 ± 40.4 [a]	59.2 ± 13.7 [b]	77.5 ± 20.4 [ab]	88.7 ± 17.2 [a]	0.003
Submucosa width (μm)	29.5 ± 4.7 [a]	31.2 ± 8.2 [a]	15.8 ± 3.2 [b]	24.6 ± 3.8 [a]	29.8 ± 6.0 [a]	<0.001
Goblet cells	1567 ± 776	1396 ± 471	845 ± 180	1509 ± 601	1612 ± 845	0.078
Neutral goblet cells	426 ± 389	224 ± 161	281 ± 170	389 ± 310	406 ± 249	0.440
Acid goblet cells	1141 ± 790	1172 ± 496	564 ± 96	1120 ± 529	1206 ± 725	0.125
Lamina propria width (μm)	19.1 ± 3.3 [a]	18.5 ± 4.9 [ab]	13.8 ± 2.1 [b]	17.4 ± 3.3 [ab]	19.6 ± 3.6 [a]	0.010
Villus length (μm)	1227.6 ± 213.1 [ab]	1238.5 ± 167.0 [a]	943.3 ± 185.5 [b]	1328.8 ± 312.3 [a]	1180.7 ± 142.6 [ab]	0.006

Values represent mean ± standard deviation ($n = 9$). In each row, different letters indicate significant differences between treatments ($p < 0.05$).

3.4. Anterior intestine histomorphology

Fish intestinal structure was well-preserved in all samples, and the diets did not lead to significant differences regarding inflammatory response of anterior intestine. *Villus* length was highest for RNF, followed by the NC diet (Fig. 3, Table 5). These values were significantly higher than the RMF diet, which displayed the shortest *villus* length. Cross-sectional perimeter and submucosa width were significantly lower for the RMF when compared to the remaining diets. *Muscularis externa* was widest for the controls, followed by AH, and these values were significantly different from the RMF diet, but not from the RNF. PC and AH diets had a significantly wider lamina propria than the RMF diet. No significant differences were found on goblet cell number.

3.5. Immune parameters

Innate immunity markers were evaluated in the plasma (Table 6). Lysozyme was lowest in fish fed RMF, being significantly different only from those fed AH and PC diets. Peroxidase was significantly reduced in the PC group, compared to the remaining ones apart from the RNF group. No significant differences were found in the ACH50.

3.6. Infectious challenge

The cumulative mortality of fish subjected to an infectious challenge with *T. maritimum* after the growth trial is depicted in Fig. 4. Fish previously fed RNF had the lowest mortality rate (<5%) that differed significantly from those fed the NC and RMF. Mortality of fish fed AH and PC did not differ from RNF. RMF had the highest cumulative mortality that did not differ significantly from fish fed the NC.

3.7. PCA analysis

The PCA plot (Fig. 5), shows the similarities and dissimilarities for the different diets and explains 76% of the data variation. Diets PC, NC and RNF are the most similar ones, while RMF is distanced, particularly on the F1 axis which explains 60% of the variation. AH is the hydrolysate diet with the highest distance from the controls on the F2 axis. PC, NC and RNF are associated with higher ADC values, body weight, nutrient gain and goblet cell number, while RMF is more associated with higher nutrient loss and FCR.

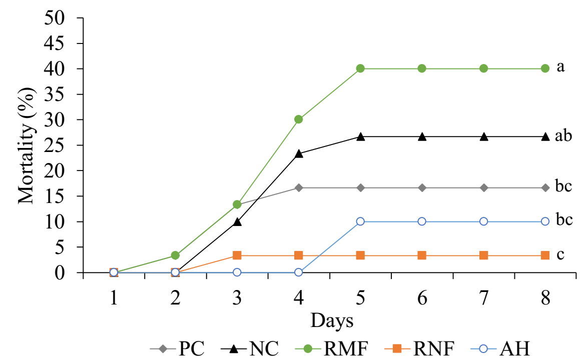

Fig. 4. Mortality rate of seabass fed the experimental diets, after a challenge with *T. maritimum*. Different lowercase letters in the graph indicate significant differences in mortality among treatments ($p < 0.05$).

4. Discussion

The dietary inclusion of hydrolysates, which may contain bioactive peptides, can minimize the negative effects associated with high levels of plant protein sources in aquafeeds (Costa et al., 2020; Gisbert et al., 2018; Gunathilaka et al., 2021). Moreover, the use of hydrolysates from coproducts of the food industry has both economic and environmental benefits, as this contributes to their valorization and simultaneously promotes a circular economy (San Martin et al., 2020). In this work, the inclusion of swine blood hydrolysates produced using an innovative approach were evaluated in European seabass. The BH were derived from a protein rich blood matrix, resulting in high levels (>91%) of crude protein and low levels of crude fat. In the FPLC analysis, RMF-H displayed a lower total area, which could indicate that this hydrolysate possesses a significant non-soluble protein fraction, compared to the remaining ones, justified by the aggregates formed during the blood heat treatment applied immediately after collection, to avoid contamination. Furthermore, the peptide profile peaks of AH are present at lower elution volumes, indicating a presence of higher sized peptide. In contrast, the RNF-H displays a peak below 1.2 kDa, at approximately 45 mL of elution, which could indicate the presence of small sized peptides and amino acids, which are not present in the remaining diets. This was expected, as AH-H was not fractionated, meaning it would contain a

Table 6
Immune parameters in the plasma of European seabass fed the experimental diets.

	Diets					*P* value
	PC	NC	RMF	RNF	AH	
Lysozyme (μg mL^{-1})	16.44 ± 4.06[a]	15.74 ± 3.10[ab]	12.26 ± 2.29[b]	14.41 ± 3.09[ab]	16.66 ± 2.03[a]	0.020
Peroxidase (EU mL^{-1})	14.43 ± 8.62[b]	33.78 ± 13.87[a]	74.88 ± 53.95[a]	33.76 ± 15.79[ab]	39.41 ± 14.42[a]	0.001
ACH50 (Units mL^{-1})	116.88 ± 24.10	167.56 ± 49.45	136.80 ± 76.21	111.57 ± 55.27	135.58 ± 75.49	0.427

Values presented as mean ± SD. In each row, different letters indicate significant differences between treatments ($p < 0.05$).

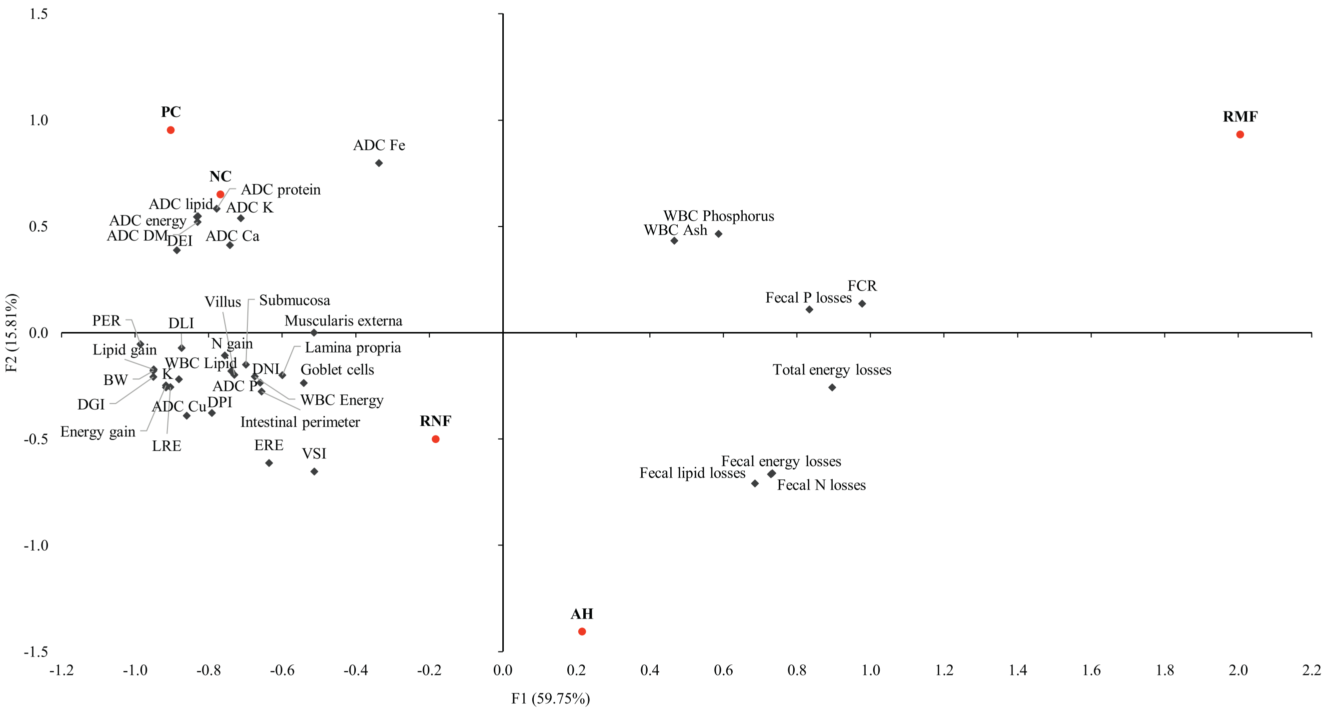

Fig. 5. Principal component analysis plot of variables of interest regarding nutrient utilization, growth and intestinal morphology. Black rhombus represent variables, while red dots represent dietary treatments. Abbreviations are: ADC – apparent digestibility coefficient; BW – final body weight; DGI – daily growth index; DLI – digestible lipid intake; DM – dry matter; DNI – digestible nitrogen intake; DPI – digestible phosphorus intake; ERE – energy retention efficiency; FCR – feed conversion ratio; K – condition index; LRE – lipid retention efficiency; PER – protein efficiency ratio; VSI – viscerosomatic index; WBC – final whole body composition. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

wide range of peptides of various sizes. In contrast, the fractionation of the enzymatic hydrolysates separates the non-soluble protein fraction in the RMF-H and concentrates the smaller sized peptides in the RNF-H. This also means that, although enzymatic hydrolysis is more specific than autohydrolysis, in which it is harder to control peptide sequence or characteristics (Álvarez-Viñas et al., 2021; Lee et al., 2021), further processing AH-H (fractionating and concentrating as for the enzymatic hydrolysate, using micro and nanofiltration) would probably lead to a high molecular peptide fraction (near 43 kDa) and a retentate from the nanofiltration with a close profile to the one of RNF-H (with some major peaks between 1.2 and 13.7 kDa, and minor but significant peaks below 1.2 kDa).

All hydrolysates showed low amounts of two EAA, methionine and isoleucine, which is in line with the values described for blood meal (National Research Council, 2011) and a blood meal hydrolysate (Xu et al., 2017). Nevertheless, all diets had a low inclusion level of each hydrolysate and were formulated to meet the amino acid requirements of European seabass. So, even considering the significant decrease of protein digestibility with the inclusion of AH-H hydrolysate, this is not expected to be an issue. Although the mineral profile of BH is seldom reported in the literature, the mineral composition of porcine blood represents approximately 4% of the dry weight (Toldrà et al., 2019). The AH-H displayed levels that were well above this value, which can be explained by the addition of sodium hydroxide in its production, resulting in its highest amount of sodium. Fractionation seems an effective way to reduce iron concentration in the hydrolysates, as RNF-H, subjected to an additional filtration step, had the lowest iron amount. To the authors' knowledge, iron removal using ultrafiltration membranes has never been attempted in blood hydrolysates but has been described in wastewaters (Choo et al., 2005; Leal et al., 2015). More importantly, all diets meet the EU maximum limit for iron (Maage et al., 2008), indicating the feasibility of aquafeeds supplementation with such BH. Mineral profiling of the diets is within the range of values described in other reports of European seabass diets (Henry et al., 2020; Siano et al., 2017).

The diets with hydrolysates had a significantly lower DM ADC compared to the PC, mainly due to the lower digestibility of lipids observed in all BH diets, and protein in AH, only. This was mainly due to the highest fecal losses of nitrogen and lipids (and, consequently, energy), as non-fecal losses remained similar among diets.

In particular, the RMF diet had a negative impact on fish growth and feed efficiency mainly due to a poorer lipid and energy retention efficiency than controls. Additionally, digestibility of calcium and copper was much poorer for this diet than for the remaining treatments. Calcium is an essential mineral to European seabass, particularly as a structural component of hard tissues, although the exact calcium needs are not yet defined for this species (National Research Council, 2011). In a study by Maas et al. (2020), Nile tilapia (*Oreochromis niloticus*) fed diets which led to reduced calcium digestibility, also showed diminished growth performance. Furthermore, PC, NC and RNF diets, which had the best growth performances, had also the highest calcium digestibility, which also indicates the importance of this mineral to proper fish growth. Copper digestibility was also lowered by the RMF diet, and it must be considered that copper plays an important role in several enzymes, such as cytochrome C oxidase, lysyl oxidase, ferroxidase, tyrosinase and superoxide dismutase, and its deficiency may also impair growth (National Research Council, 2011). Although these activities have not been presently studied, it is visible from the PCA plot that the ADC of copper is associated with lipid gain, and the RMF diet is negatively associated with these variables. In fact, an association between higher copper digestibility and availability and an improved lipid absorption and metabolism has been described in piglets, and authors suggested copper enhances lipase activity and modulates genes involved in the post-absorptive metabolism of lipids (Espinosa and Stein, 2021; Gonzales-Eguia et al., 2009).

Furthermore, RMF diet displayed several alterations in the anterior intestine morphology. The most evident ones are a decrease of the *villus* length and *muscularis externa* width. It has been described that a reduction in these parameters can be linked to lower nutrient uptake, leading to a reduction in nutrient digestibility and fish growth (Batista et al., 2020; Valente et al., 2021). Thus, results suggest that the impact of RMF-H on the intestinal morphology could at least partially explain the poorer overall performance of the diet with this BH.

In general, the remaining diets did not differ in most of the parameters evaluated in the anterior intestine which are within the range of values described in the literature for this species (Batista et al., 2020, Valente et al., 2021). Moreover, despite the larger submucosa width in fish fed the PC, NC, RNF and AH diets, when compared to RMF, this does not seem to be associated with any inflammatory response, as no differences were seen in the cellularity or presence of inflammatory infiltrates among all diets. The impact of several bioactive hydrolysates on intestine morphology has been reported on literature and varies greatly. In the work of Leduc et al. (2018b), the inclusion of shrimp hydrolysate in diets for European seabass resulted in larger *villi* and more goblet cells, when compared to a high FM diet. However, the same authors found no differences in those parameters when using a tilapia hydrolysate, which once again showed the variability of the impact of hydrolysates originated from distinct sources or submitted to different processing. Egerton et al. (2020) found that the inclusion of soluble fish protein hydrolysate in Atlantic salmon diets increased posterior intestine walls and *villi* width, but without repercussions in fish growth. However, a partly insoluble hydrolysate did not affect intestine morphology.

The role of bioactive hydrolysates on diet digestibility is not frequently reported in the literature. Most reports evaluated hydrolysates as FM replacement, testing higher inclusion levels than those used in the present study. Thus, results are often contradictory. Zheng et al. (2012) claimed that both an ultra-filtered and a non-filtered fish hydrolysate (at 37 g kg^{-1}) improved DM and protein ADC in Japanese flounder (*Paralichthys olivaceus*). In contrast, Khosravi et al. (2015) observed no differences in the DM ADC of red sea bream (*Pagrus major*) diets supplemented with 2% of a tuna or krill hydrolysate. In the present study, RNF was equally well digested as the NC diet, resulting in the lowest fecal nutrient losses of all studied BH and being the most adequate hydrolysate to prevent effluent nutrient load. This could be due to the presence of lower sized peptides (below 1.2 kDa), concentrated in the RNF-H fraction (Araújo-Rodrigues et al., 2022), that may be more easily absorbed and provide better bioactive properties. Zheng et al. (2012) noticed that an ultra-filtered hydrolysate with smaller peptides (0.1–1 kDa) led to higher protein and DM ADC in Japanese flounder than a non-ultra-filtered (>10 kDa) fish hydrolysate. Likewise, the peptides present in the RNF-H fraction also displayed a peak below 1.2 kDa which could play an important role in improving protein digestibility.

Mineral digestibility is not often evaluated in marine species, due to the possible interference of waterborne minerals in the analysis (Storebakken, 2000). In any case, phosphorus digestibility values are in line with others reported in the literature for this species (Ballester-Moltó et al., 2017; Magalhães et al., 2018; Valente et al., 2021). The RNF diet led to the lowest level of fecal losses and increased digestible phosphorus, achieving better results than the PC. This could indicate a lower environmental impact of this diet due to the lesser contamination of effluents with phosphorus loads (Ballester-Moltó et al., 2017; Campos et al., 2017).

Regarding the ADC values of the remaining minerals, they are generally within the range found by Sugiura et al. (1998) for the rainbow trout (*Oncorhynchus mykiss*) and Coho salmon (*Oncorhynchus kisutch*), except for calcium and potassium ADC, which were substantially higher and lower, respectively, in the present work. It should be noted that differences could be a result of variability among species, as well as different diet formulation, as the authors also found a high variability in mineral digestibility among several common aquafeed ingredients. Moreover, potassium ADC in this work could be underestimated due to some interference of waterborne potassium in feces analysis (Storebakken, 2000), which can justify the lower ADC values; such interference is not expected in freshwater, used in the above cited works. Nonetheless, as the water conditions were equal among the whole RAS system, it is expected that they affected ADCs in a similar way, meaning that differences among diets should still be valid and due to the diet itself. RNF did not show any significant differences in any mineral ADC, suggesting it does not impair mineral nutrition. AH lowered iron digestibility compared to both controls, but this is not surprising as this diet had the highest iron levels, suggesting that the maximum capacity of fish to absorb iron was reached, with the remaining being lost in feces.

Although growth performance and feed efficacy indicators were within the range of values reported in the literature for European seabass juveniles (Campos et al., 2017; Campos et al., 2019; Leduc et al., 2018a), the dietary inclusion of the hydrolysates affected the seabass' growth performance. The PC, a diet with a high content of FM, led to a better growth performance than the NC, a diet rich in vegetable protein sources, demonstrating how challenging it can be to replace FM in this carnivorous species even when nutrient requirements are met. The negative impact of FM replacement by plant proteins on growth has also been described by other authors (Dias et al., 2005; Wassef et al., 2019). In spite of this, RNF was the hydrolysate with the best results, showing no significant differences in daily growth index or feed efficiency compared to the PC. RNF also led to the longest *villus*, which could indicate good nutrient absorption capacity facilitating growth (Batista et al., 2020). This can once more be attributed to the lower sized peptides of the RNF-H.

It has been described that when comparing different size fractions of fish hydrolysate for diets for Atlantic cod (*Gadus morhua*), smaller sized molecules (<0.1 kDa) displayed an important role in fish growth and feed utilization (Aksnes et al., 2006). Costa et al. (2020) and Leduc et al. (2018b) supplemented plant-based diets with selected marine hydrolysates and achieved seabass final weight comparable to that of fish fed a FM-based diet. Nevertheless, Costa et al. (2020) also showed that the impact on European seabass growth performance differed both with marine hydrolysate sources and enzymatic processing. The hydrolysates used in these works were obtained from sources and processes that greatly differ from the processing of swine blood herein described, and therefore the resulting peptides are expected to be very different, in terms of size, AA profile and bioactivities. In a previous study that applied swine blood hydrolysates in microdiets for seabream larvae, growth was also lower than that observed with diets rich in FM, albeit not significantly (Gisbert et al., 2012). Fernández-Alacid et al. (2021) also reported an impairment in growth of meagre fed diets with a replacement of FM with porcine plasma hydrolysates (with an inclusion level of 5%). Another work with turbot (*Scophthalmus maximus*) has also shown that a diet with 20% plant protein and 8% pig blood hydrolysate led to a reduced growth performance when compared to a diet with 30% FM (Xu et al., 2017). Additionally, in that work, fish fed the BH displayed higher HSI and increased the activities of serum transaminases, which suggests liver damage, a factor that could impair fish growth. In the present study, no differences were found on HSI, which negates the idea there was liver damage caused by diets. It has also been suggested that hydrolysate inclusion increases mRNA transcripts of insulin-like growth factor I (IGF) in liver and plasma of Japanese flounder fed with pollock by-product hydrolysates (Zheng et al., 2012). Gui et al. (2010) have found that supplementation of plant-based diets for crucian carp with cottonseed hydrolysates diminished the IGF levels for inclusion ratios of 100 g kg^{-1}, even if this was not reflected in final weight. In order to fully understand the impact of BP in the European sea bass metabolic processes the expression of genes linked to fish growth, such as IGF, should be also deeply investigated. Although lipid gain and retention efficiency were lowest for RMF, the whole-body composition did not differ significantly among dietary treatments. A trend was, however, noted for a reduction in the lipid composition of fish fed RMF, that might have an impact in a longer feeding trial, considering increased lipid deposition with fish age. In a previous study with European seabass juveniles, Costa et al. (2020) also found no effect of functional marine hydrolysates in fish final composition. In addition, Novriadi et al. (2017) studied the inclusion of up to 4% of squid hydrolysates in diets for Florida pompano *Trachinotus carolinus* and found that this did not affect fish proximate composition, but only the mineral fish profile. In our work, the RMF diet did in fact slightly increase whole-body phosphorus content, but not significantly.

The replacement of FM with plant protein sources may also impact fish immune status, as described by Azeredo et al. (2017), Conde-Sieira et al. (2018) and Xu et al. (2019). In the present study, we have selected three common indicators of fish innate immunity, which are fully functional since hatching, unlike the adaptive immunity, which has a later onset (Galindo-Villegas and Mulero, 2014; Swain and Nayak, 2009). Furthermore, they have a broader spectrum of action, being good indicators of overall fish immune status (Alvarez-Pellitero, 2008). Lysozyme hydrolyzes peptidoglycan residues in bacterial cell walls (Buonocore et al., 2014), whilst peroxidase is involved in the oxidative response against pathogens (Alvarez-Pellitero, 2008). 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). The PC diet displayed lower plasmatic peroxidase activity, but the remaining parameters were similar to those of the NC. The hydrolysates did not increase significantly the innate immune parameters in the plasma, compared to the NC. In contrast, Costa et al. (2020) found that the inclusion of marine hydrolysates in diets for European seabass raised lysozyme levels above those of a plant-based diet, reaching similar values to those fed the FM-based diet. Moreover, Gisbert et al. (2018) observed that the inclusion of shrimp hydrolysates in a low FM diet increased the non-specific immunity indicators to values above those induced by a FM-based diet. According to Leduc et al. (2018b), a shrimp hydrolysate displayed stronger immunostimulant properties than a tilapia hydrolysate, when included at the same level in diets for European seabass juveniles. Once more, the authors propose that the raw material used to manufacture hydrolysates is an important determinant of the outcomes, and this could help explain the differences with the results here presented.

Despite the apparent lack of innate immune parameters modulation by the BH, a significant increase in the resistance to a challenge with *T. maritimum* was provided by the RNF diet, when compared to the NC diet. The mortality of the plant-based NC was slightly higher than the PC, but the difference was not statistically significant. However, when 3% of the RNF-H is added to a plant-based diet, mortality decreases significantly from the NC and actually falls below that of the FM based diet (even if not significantly). This is a major achievement, considering that this infection has become a serious problem in aquaculture farms for several species (Gourzioti et al., 2016; Khalil et al., 2018; Mabrok et al., 2016; Valdes et al., 2021). Diets with AH-H also seem to be promising in this matter, and they could facilitate process feasibility, but further tests should be made with other AH-fractions (eventually from nanofiltration) to validate and minimize the impacts on growth and digestibility. To the best of our knowledge, the *in vivo* evaluation of functional diets (particularly hydrolysates) for prevention of *T. maritimum* is scarce, although micro- and macroalgae *in vitro* antibacterial activity against this bacteria has been recently evidenced (Ferreira et al., 2021). The inclusion of 5% shrimp hydrolysate in diets for European seabass juveniles led to better survival rates, after an outbreak of *Vibrio pelagius*, regardless of the dietary FM level (Gisbert et al., 2018). However, in Atlantic cod, Gildberg and Mikkelsen (1998) found that the inclusion of immunostimulating peptides (by spraying 35 mL of peptide solution into 200 g of diet) did not improve fish resistance when challenged with *V. anguillarum*, although the infection dosage was apparently higher than the presence of moderate levels of opportunistic bacteria frequently seen in fish farms. Moreover, in European seabass larvae, the dose of hydrolysates does not seem to play a very important role on mortality after infection with *V. anguillarum*, contrarily to the type of hydrolysate – commercial fish hydrolysate vs experimental sardine hydrolysate (Kotzamanis et al., 2007). The reasons behind the effectiveness of hydrolysates on the control of infections can vary: upregulation of hepcidin expression, with antifungal and antibacterial activity (Gong et al., 2019); improved non-specific humoral immunity (Gisbert et al., 2018); changes in blood glucose and micro-morphology of the intestine (Siddik et al., 2018); or microbiota alterations, promoting an antagonistic environment preventing the evolution of pathogens (Kotzamanis et al., 2007). In the present study, the RNF increased resistance to *T. maritimum* without providing significant intestinal morphological or innate immunity improvements. In contrast, the RMF decreased intestinal health and was also responsible for lower lysozyme plasma levels, which could explain the observed lower resistance. Gut microbiota was not presently assessed, so further studies should be performed to better clarify the mechanism underlying the BH responses.

5. Conclusions

The RNF-H stood out as a promising hydrolysate to be added to plant-based diets for European seabass, since its dietary inclusion, at 3%, led to an increased resistance towards *T. maritimum*. This was achieved without impairing fish growth or nutrient utilization, compared to the control plant-based diet. Considering the negative impact of *T. maritimum* on fish health and its frequent outbreaks in fish farms, along with the absence of a vaccine for many fish species, the results herein presented suggest that this hydrolysate could be a good strategy to prevent fish tenacibaculosis and further economic damage in fish farms. Moreover, the hydrolysate production follows a circular economy approach, as the raw material is a coproduct of another industry (meat industry). More studies should be further performed to identify the peptides and immunological pathways underlying the observed increased fish protection towards infection.

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CRediT authorship contribution statement

Daniela Resende: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Benjamin Costas:** Conceptualization, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Tiago Sá:** Formal analysis, Investigation, Validation, Writing – review & editing. **Umberto Golfetto:** Formal analysis, Investigation, Validation, Writing – review & editing. **Marina Machado:** Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing. **Miguel Pereira:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Carlos Pereira:** Conceptualization, Methodology, Resources, Writing – review & editing. **Bianca Marques:** Methodology, Resources, Writing – review & editing. **Cristina M.R. Rocha:** Conceptualization, Methodology, Resources, Writing – review & editing. **Manuela Pintado:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Luisa M.P. Valente:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Adhikari, B.B., Chae, M., Bressler, D.C., 2018. Utilization of slaughterhouse waste in value-added applications: recent advances in the development of wood adhesives. Polymers (Basel). 10, 176. https://doi.org/10.3390/polym10020176.

Aiemratchanee, P., Panyawechamontri, K., Phaophu, P., Reamtong, O., Panbangred, W., 2021. *In vitro* antihypertensive activity of bioactive peptides derived from porcine blood corpuscle and plasma proteins. Int. J. Food Sci. Technol. 56, 2315–2324. https://doi.org/10.1111/ijfs.14853.

Aksnes, A., Hope, B., Høstmark, Ø., Albrektsen, S., 2006. Inclusion of size fractionated fish hydrolysate in high plant protein diets for Atlantic cod, *Gadus morhua*. Aquaculture. 261, 1102–1110. https://doi.org/10.1016/j.aquaculture.2006.07.038.

Alvarez-Pellitero, P., 2008. Fish immunity and parasite infections: from innate immunity to immunoprophylactic prospects. Vet. Immunol. Immunopathol. 126, 171–198. https://doi.org/10.1016/j.vetimm.2008.07.013.

Álvarez-Viñas, M., Rodríguez-Seoane, P., Flórez-Fernández, N., Torres, M.D., Díaz-Reinoso, B., Moure, A., Domínguez, H., 2021. Subcritical water for the extraction and hydrolysis of protein and other fractions in biorefineries from agro-food wastes and algae: a review. Food Bioprocess Technol. 14, 373–387. https://doi.org/10.1007/s11947-020-02536-4.

Araújo-Rodrigues, H., Coscueta, E.R., Pereira, M.F., Cunha, S.A., Almeida, A., Rosa, A., Martins, R., Pereira, C.D., Pintado, M.E., 2022. Membrane fractionation of *Cynara cardunculus* swine blood hydrolysate: ingredients of high nutritional and nutraceutical value. Food Res. Int. 128, 111549. https://doi.org/10.1016/j.foodres.2022.111549.

Aubin, J., Callier, M., Rey-Valette, H., Mathé, S., Wilfart, A., Legendre, M., Fontaine, P., 2019. Implementing ecological intensification in fish farming: definition and principles from contrasting experiences. Rev. Aquac. 11, 149–167. https://doi.org/10.1111/raq.12231.

Azeredo, R., Machado, M., Kreuz, E., Wuertz, S., Oliva-Teles, A., Enes, P., Costas, B., 2017. The European seabass (*Dicentrarchus labrax*) innate immunity and gut health are modulated by dietary plant-protein inclusion and prebiotic supplementation. Fish Shellfish Immunol. 60, 78–87. https://doi.org/10.1016/j.fsi.2016.11.019.

Bah, C.S.F., Bekhit, A.E.-D.A., Carne, A., McConnell, M.A., 2013. Slaughterhouse blood: an emerging source of bioactive compounds. Compr. Rev. Food Sci. Food Saf. 12, 314–331. https://doi.org/10.1111/1541-4337.12013.

Ballester-Moltó, M., Follana-Berná, G., Sanchez-Jerez, P., Aguado-Giménez, F., 2017. Total nitrogen, carbon and phosphorus digestibility in gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) fed with conventional and organic commercial feeds: implications for particulate waste production. Aquac. Res. 48, 3450–3463. https://doi.org/10.1111/are.13171.

Batista, S., Pereira, R., Oliveira, B., Baião, L.F., Jessen, F., Tulli, F., Valente, L.M.P., 2020. Exploring the potential of seaweed *Gracilaria gracilis* and microalga *Nannochloropsis oceanica*, single or blended, as natural dietary ingredients for European seabass *Dicentrarchus labrax*. J. Appl. Phycol. 32, 2041–2059. https://doi.org/10.1007/s10811-020-02118-z.

Boshra, H., Li, J., Sunyer, J.O., 2006. Recent advances on the complement system of teleost fish. Fish Shellfish Immunol. 20, 239–262. https://doi.org/10.1016/j.fsi.2005.04.004.

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. 418-419, 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.). Mol. Immunol. 62, 10–18. https://doi.org/10.1016/j.molimm.2014.05.009.

Campos, I., Matos, E., Marques, A., Valente, L.M.P., 2017. Hydrolyzed feather meal as a partial fishmeal replacement in diets for European seabass (*Dicentrarchus labrax*) juveniles. Aquaculture. 476, 152–159. https://doi.org/10.1016/j.aquaculture.2017.04.024.

Campos, I., Matos, E., Maia, M.R.G., Marques, A., Valente, L.M.P., 2019. Partial and total replacement of fish oil by poultry fat in diets for European seabass (*Dicentrarchus labrax*) juveniles: effects on nutrient utilization, growth performance, tissue composition and lipid metabolism. Aquaculture. 502, 107–120. https://doi.org/10.1016/j.aquaculture.2018.12.004.

Castro, L.M.G., Ribeiro, T.B., Machado, M., Alexandre, E.M.C., Saraiva, J.A., Pintado, M., 2022. Unraveling the effect of dehulling methods on the nutritional composition of acorn *Quercus* spp. J. Food Compos. Anal. 106, 104354 https://doi.org/10.1016/j.jfca.2021.104354.

Cho, C.Y., Slinger, S.J., 1979. Apparent digestibility measurement in feedstuffs for rainbow trout. In: Halver, J.E., Tiews, K. (Eds.), Finfish Nutrition and Fish Feed Technology. Heeneman, Berlin, pp. 239–247.

Choo, K.-H., Lee, H., Choi, S.-J., 2005. Iron and manganese removal and membrane fouling during UF in conjunction with prechlorination for drinking water treatment. J. Membr. Sci. 267, 18–26. https://doi.org/10.1016/j.memsci.2005.05.021.

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

Costa, M., Costas, B., Machado, M., Teixeira, C., Fernández-Boo, S., Sá, T., 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. Int. J. Fish Aquat. Stud. 6, 164–179.

Daroit, D.J., Brandelli, A., 2021. *In vivo* bioactivities of food protein-derived peptides – a current review. Curr. Opin. Food Sci. 39, 120–129. https://doi.org/10.1016/j.cofs.2021.01.002.

Defoirdt, T., Sorgeloos, P., Bossier, P., 2011. Alternatives to antibiotics for the control of bacterial disease in aquaculture. Curr. Opin. Microbiol. 14, 251–258. https://doi.org/10.1016/j.mib.2011.03.004.

Dias, J., Alvarez, M.J., Arzel, J., Corraze, G., Diez, A., Bautista, J.M., Kaushik, S.J., 2005. Dietary protein source affects lipid metabolism in the European seabass (*Dicentrarchus labrax*). Comp. Biochem. Physiol. A 142, 19–31. https://doi.org/10.1016/j.cbpb.2005.07.005.

Egerton, S., Wan, A., Murphy, K., Collins, F., Ahern, G., Sugrue, I., Stanton, C., 2020. Replacing fishmeal with plant protein in Atlantic salmon (*Salmo salar*) diets by supplementation with fish protein hydrolysate. Sci. Rep. 10, 4194. https://doi.org/10.1038/s41598-020-60325-7.

Espinosa, C.D., Stein, H.H., 2021. Digestibility and metabolism of copper in diets for pigs and influence of dietary copper on growth performance, intestinal health, and overall immune status: a review. J. Animal Sci. Biotechnol. 12, 13. https://doi.org/10.1186/s40104-020-00533-3.

European Commission, 2020. Farm to Fork Strategy. Retrieved from. https://ec.europa.eu/food/system/files/2020-05/f2f_action-plan_2020_strategy-info_en.pdf. on 02-12-2021.

European Commission, 2021. Pigmeat statistics. Retrieved from. https://ec.europa.eu/food-farming-fisheries/farming/facts-and-figures/markets/overviews/market-observatories/meat/pigmeat-statistics_en. on 08-07-2021.

European Union, 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes, L276. European Union (Official Journal of the European Union).

Faustino, M., Veiga, M., Sousa, P., Costa, E.M., Silva, S., Pintado, M., 2019. Agro-food byproducts as a new source of natural food additives. Molecules. 24, 1056. https://doi.org/10.3390/molecules24061056.

Fernández-Alacid, L., Firmino, J.P., Sanahuja, I., Madrid, C., Polo, J., de Borba, M.R., Ibarz, A., 2021. Impact of dietary porcine blood by-products in meagre (*Argyrosomus regius*) physiology, evaluated by welfare biomarkers and the antibacterial properties of the skin mucus. Fish Shellfish Immunol. 118, 241–250. https://doi.org/10.1016/j.fsi.2021.09.011.

Ferreira, M., Teixeira, C., Abreu, H., Silva, J., Costas, B., Kiron, V., Valente, L.M.P., 2021. Nutritional value, antimicrobial and antioxidant activities of micro- and macroalgae, single or blended, unravel their potential use for aquafeeds. J. Appl. Phycol. 33, 3507–3518. https://doi.org/10.1007/s10811-021-02549-2.

Galindo-Villegas, J., Mulero, V., 2014. Current knowledge on the development and functionality of immune responses in the European sea bass (*Dicentrarchus labrax*). In: Vázquez, F.J.S., Muñoz-Cueto, J.A. (Eds.), Biology of European Sea Bass. CRC Press, Boca Raton, pp. 342–373.

Ghalamara, S., Silva, S., Brazinha, C., Pintado, M., 2020. Valorization of fish by-products: purification of bioactive peptides from codfish blood and sardine cooking wastewaters by membrane processing. Membranes. 10, 44. https://doi.org/10.3390/membranes10030044.

Gildberg, A., Mikkelsen, H., 1998. Effects of supplementing the feed to Atlantic cod (*Gadus morhua*) fry with lactic acid bacteria and immuno-stimulating peptides during a challenge trial with *vibrio anguillarum*. Aquaculture. 167, 103–113. https://doi.org/10.1016/S0044-8486(98)00296-8.

Gisbert, E., Skalli, A., Fernández, I., Kotzamanis, Y., Zambonino-Infante, J.L., Fabregat, R., 2012. Protein hydrolysates from yeast and pig blood as alternative raw materials in microdiets for gilthead sea bream (*Sparus aurata*) larvae. Aquaculture. 338-341, 96–104. https://doi.org/10.1016/j.aquaculture.2012.01.007.

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.

Gisbert, E., Ibarz, A., Firmino, J.P., Fernández-Alacid, L., Salomón, R., Vallejos-Vidal, E., Andree, K.B., 2021. Porcine protein hydrolysates (PEPTEIVA®) promote growth and enhance systemic immunity in gilthead sea bream (*Sparus aurata*). Animals. 11, 2122. https://doi.org/10.3390/ani11072122.

Gong, Y., Yang, F., Hu, J., Liu, C., Liu, H., Han, D., Xie, S., 2019. Effects of dietary yeast hydrolysate on the growth, antioxidant response, immune response and disease resistance of largemouth bass (*Micropterus salmoides*). Fish Shellfish Immunol. 94, 548–557. https://doi.org/10.1016/j.fsi.2019.09.044.

Gonzales-Eguia, A., Fu, C.-M., Lu, F.-Y., Lien, T.-F., 2009. Effects of nanocopper on copper availability and nutrients digestibility, growth performance and serum traits of piglets. Livest. Sci. 126, 122–129. https://doi.org/10.1016/j.livsci.2009.06.009.
Gourzioti, E., Kolygas, M.N., Athanassopoulou, F., Babili, V., 2016. Tenacibaculosis in aquaculture farmed marine fish. J. Hell. Vet. Med. Soc. 67, 21–32. https://doi.org/10.12681/jhvms.15620.
Gudding, R., Van Muiswinkel, W.B., 2013. A history of fish vaccination: science-based disease prevention in aquaculture. Fish Shellfish Immunol. 35, 1683–1688. https://doi.org/10.1016/j.fsi.2013.09.031.
Gui, D., Liu, W., Shao, X., Xu, W., 2010. Effects of different dietary levels of cottonseed meal protein hydrolysate on growth, digestibility, body composition and serum biochemical indices in crucian carp (*Carassius auratus gibelio*). Anim. Feed Sci. Technol. 156, 112–120. https://doi.org/10.1016/j.anifeedsci.2010.01.012.
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*). Fish. Aquat. Sci. 24, 109–120. https://doi.org/10.47853/FAS.2021.e11.
Hardy, R.W., Barrows, F.T., 2002. Diet formulation and manufacture. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Academic Press, San Diego, pp. 505–600.
Henry, M.A., Fountoulaki, E., Vasilaki, A., Rigos, G., Kokou, F., Karalazos, V., 2020. Dietary micronutrient supplementation in low fishmeal based diets for optimum growth and immune status of European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture. 528, 735479 https://doi.org/10.1016/j.aquaculture.2020.735479.
Hua, K., Cobcroft, J.M., Cole, A., Condon, K., Jerry, D.R., Mangott, A., Strugnell, J.M., 2019. The future of aquatic protein: implications for protein sources in aquaculture diets. One Earth. 1, 316–329. https://doi.org/10.1016/j.oneear.2019.10.018.
ISO 13730, 1996. Meat and Meat Products — Determination of Total Phosphorus Content — Spectrometric Method. Retrieved from. https://www.iso.org/standard/22789.html.
Khalil, R.H., Diab, A.M., Shakweer, M.S., Ghetas, H.A., Khallaf, M.M., Omar, A., 2018. New perspective to control of tenacibaculosis in sea bass *Dicentrarchus labrax* L. Aquac. Res. 49, 2357–2365. https://doi.org/10.1111/are.13689.
Khosravi, S., Bui, H.T.D., Rahimnejad, S., Herault, M., Fournier, V., Kim, S.-S., Lee, K.-J., 2015. Dietary supplementation of marine protein hydrolysates in fish-meal based diets for red sea bream (*Pagrus major*) and olive flounder (*Paralichthys olivaceus*). Aquaculture. 435, 371–376. https://doi.org/10.1016/j.aquaculture.2014.10.019.
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., Zambonino Infante, J., 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. Comp. Biochem. Phys. A. 147, 205–214. https://doi.org/10.1016/j.cbpa.2006.12.037.
Krogdahl, Bakke-Mckellep, RØed, Baeverfjord, 2000. Feeding Atlantic salmon *Salmo salar* L. soybean products: effects on disease resistance (furunculosis), and lysozyme and IgM levels in the intestinal mucosa. Aquac. Nutr. 6, 77–84. https://doi.org/10.1046/j.1365-2095.2000.00129.x.
Leal, M.J.C., do Amaral, P.A.P., Nagel-Hassemer, M.E., Lobo-Recio, M.Á., Lapolli, F.R., 2015. Aquatic humic substances, iron, and manganese removal by ultrafiltration and nanofiltration membranes combined with coagulation–flocculation–sedimentation. Desalin. Water Treat. 55, 1662–1671. https://doi.org/10.1080/19443994.2015.1012337.
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., 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, 396. https://doi.org/10.1186/s12864-018-4780-0.
Lee, H.-J., Roy, V.C., Ho, T.C., Park, J.-S., Jeong, Y.-R., Lee, S.-C., Chun, B.-S., 2021. Amino acid profiles and biopotentiality of hydrolysates obtained from comb penshell (*Atrina pectinata*) viscera using subcritical water hydrolysis. Mar. Drugs. 19, 137. https://doi.org/10.3390/md19030137.
Maage, A., Hove, H., Julshamn, K., 2008. 22 - monitoring and surveillance to improve farmed fish safety. In: Lie, Ø. (Ed.), Improving Farmed Fish Quality and Safety. Woodhead Publishing, Cambridge, pp. 547–564.
Maas, R.M., Verdegem, M.C.J., Stevens, T.L., Schrama, J.W., 2020. Effect of exogenous enzymes (phytase and xylanase) supplementation on nutrient digestibility and growth performance of Nile tilapia (*Oreochromis niloticus*) fed different quality diets. Aquaculture. 529, 735723 https://doi.org/10.1016/j.aquaculture.2020.735723.
Mabrok, M., Machado, M., Serra, C.R., Afonso, A., Valente, L.M.P., Costas, B., 2016. Tenacibaculosis induction in the Senegalese sole (*Solea senegalensis*) and studies of *Tenacibaculum maritimum* survival against host mucus and plasma. J. Fish Dis. 39, 1445–1455. https://doi.org/10.1111/jfd.12483.
Magalhães, R., Díaz-Rosales, P., Diógenes, A.F., Enes, P., Oliva-Teles, A., Peres, H., 2018. Improved digestibility of plant ingredient-based diets for European seabass (*Dicentrarchus labrax*) with exogenous enzyme supplementation. Aquac. Nutr. 24, 1287–1295. https://doi.org/10.1111/anu.12666.
National Research Council, 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC.
Naylor, R.L., Hardy, R.W., Buschmann, A.H., Bush, S.R., Cao, L., Klinger, D.H., Troell, M., 2021. A 20-year retrospective review of global aquaculture. Nature. 591, 551–563. https://doi.org/10.1038/s41586-021-03308-6.
Novriadi, R., Spangler, E., Rhodes, M., Hanson, T., Allen Davis, D., 2017. Effects of various levels of squid hydrolysate and squid meal supplementation with enzyme-treated soy on growth performance, body composition, serum biochemistry and histology of Florida pompano *Trachinotus carolinus*. Aquaculture. 481, 85–93. https://doi.org/10.1016/j.aquaculture.2017.08.032.
San Martin, D., Orive, M., Iñarra, B., Castelo, J., Estévez, A., Nazzaro, J., Zufía, J., 2020. Brewers' spent yeast and grain protein hydrolysates as second-generation feedstuff for aquaculture feed. Waste Biomass Valor. 11, 5307–5320. https://doi.org/10.1007/s12649-020-01145-8.
Santos, L., Ramos, F., 2018. Antimicrobial resistance in aquaculture: current knowledge and alternatives to tackle the problem. Int. J. Antimicrob. Agents 52, 135–143. https://doi.org/10.1016/j.ijantimicag.2018.03.010.
Siano, F., Bilotto, S., Nazzaro, M., Russo, G.L., Di Stasio, M., Volpe, M.G., 2017. Effects of conventional and organic feed on the mineral composition of cultured European sea bass (*Dicentrarchus labrax*). Aquac. Nutr. 23, 796–804. https://doi.org/10.1111/anu.12446.
Siddik, M.A.B., 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*. Sci. Rep. 8, 15942. https://doi.org/10.1038/s41598-018-34182-4.
Siddik, M.A.B., Pham, H.D., Francis, D.S., Vo, B.V., Shahjahan, M., 2021. Dietary supplementation of fish protein hydrolysate in high plant protein diets modulates growth, liver and kidney health, and immunity of barramundi (*Lates calcarifer*). Aquac. Nutr. 27, 86–98. https://doi.org/10.1111/anu.13404.
Singh, M., Barman, A.S., Devi, A.L., Devi, A.G., Pandey, P.K., 2019. Iron mediated hematological, oxidative and histological alterations in freshwater fish *Labeo rohita*. Ecotoxicol. Environ. Saf. 170, 87–97. https://doi.org/10.1016/j.ecoenv.2018.11.129.
Storebakken, Shearer, Roem, 2000. Growth, uptake and retention of nitrogen and phosphorus, and absorption of other minerals in Atlantic salmon *Salmo salar* fed diets with fish meal and soy–protein concentrate as the main sources of protein. Aquac. Nutr. 6, 103–108. https://doi.org/10.1046/j.1365-2095.2000.00135.x.
Sugiura, S.H., Dong, F.M., Rathbone, C.K., Hardy, R.W., 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture. 159, 177–202. https://doi.org/10.1016/S0044-8486(97)00177-4.
Swain, P., Nayak, S.K., 2009. Role of maternally derived immunity in fish. Fish Shellfish Immunol. 27, 89–99. https://doi.org/10.1016/j.fsi.2009.04.008.
Teodósio, R., Aragão, C., Colen, R., Carrilho, R., Dias, J., Engrola, S., 2021. A nutritional strategy to promote gilthead seabream performance under low temperatures. Aquaculture. 537, 736494 https://doi.org/10.1016/j.aquaculture.2021.736494.
Toldrà, M., Lynch, S.A., Couture, R., Álvarez, C., 2019. Chapter 5 - blood proteins as functional ingredients. In: Galanakis, C.M. (Ed.), Sustainable Meat Production and Processing. Academic Press, London, pp. 85–101.
Torrecillas, S., Caballero, M.J., Mompel, D., Montero, D., Zamorano, M.J., Robaina, L., 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 Shellfish Immunol. 67, 302–311. https://doi.org/10.1016/j.fsi.2017.06.022.
Valdes, S., Irgang, R., Barros, M.C., Ilardi, P., Saldarriaga-Córdoba, M., Rivera–Bohle, J., Avendaño-Herrera, R., 2021. First report and characterization of *Tenacibaculum maritimum* isolates recovered from rainbow trout (*Oncorhynchus mykiss*) farmed in Chile. J. Fish Dis. 44, 1481–1490. https://doi.org/10.1111/jfd.13466.
Valente, L.M.P., Batista, S., Ribeiro, C., Pereira, R., Oliveira, B., Garrido, I., Kiron, V., 2021. Physical processing or supplementation of feeds with phytogenic compounds, alginate oligosaccharide or nucleotides as methods to improve the utilization of *Gracilaria gracilis* by juvenile European seabass (*Dicentrarchus labrax*). Aquaculture. 530, 735914 https://doi.org/10.1016/j.aquaculture.2020.735914.
Voss, G.B., Osorio, H., Valente, L.M.P., Pintado, M.E., 2019. Impact of thermal treatment and hydrolysis by Alcalase and *Cynara cardunculus* enzymes on the functional and nutritional value of Okara. Process Biochem. 83, 137–147. https://doi.org/10.1016/j.procbio.2019.05.010.
Wassef, Elham A., Saleh, Norhan E., Ashry, Ahmed M., Abdel-Momen, Shaban, Al-Zayat, Ahmed M., 2019. European seabass (*Dicentrarchus labrax*) performance, health status, immune response and intestinal morphology after feeding a mixture of plant proteins-containing diets. Egypt. J. Aquat. Biol. and Fish. 23, 77–91. https://doi.org/10.21608/EJABF.2019.52408.
Xu, H., Mu, Y., Liang, M., Zheng, K., Wei, Y., 2017. Application of different types of protein hydrolysate in high plant protein diets for juvenile turbot (*Scophthalmus maximus*). Aquac. Res. 48, 2945–2953. https://doi.org/10.1111/are.13127.
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 Physiol. Biochem. 45, 1331–1342. https://doi.org/10.1007/s10695-019-00641-6.
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*). Aquac. Nutr. 18, 297–303. https://doi.org/10.1111/j.1365-2095.2011.00896.x.

3.2. Stress response of European seabass (*Dicentrarchus labrax*) fed plant-based diets supplemented with swine blood hydrolysates

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Stress response of European seabass (*Dicentrarchus labrax*) fed plant-based diets supplemented with swine blood hydrolysates

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A B S T R A C T

To improve fish welfare, it is essential that aquafeeds are designed to help fish cope with the stressful conditions of fish farms. One effective strategy to achieve this goal is to supplement the diet with bioactive hydrolysates. Here, diet supplementation to modulate oxidative stress after air exposure was investigated in European seabass, using swine blood hydrolysates (BH), obtained either by autohydrolysis (AH) or enzymatically. The enzymatically produced BH were further submitted to a micro- (RMF) and nanofiltration (RNF). Four isolipidic, isoproteic and isoenergetic diets were developed: a plant-based diet with low (12.5%) fishmeal levels (control, CTRL) and three diets where 3% of each BH (RMF, RNF and AH) was added to the CTRL. Diets were assigned to triplicate groups of 71 European seabass juveniles (initial weight 12.3 ± 1.4 g). After 12 weeks, 9 fish per treatment were either immediately sampled or air-exposed for 1 min and let to recover in a new system for 6 h prior to sampling. Stress response increased cortisol levels, followed by an increment in plasma lactate. The challenge increased liver lipid peroxidation (LPO) due to reactive oxygen species (ROS) accumulation. Carbonyls decreased post-stress, maybe due to a possible interaction with the LPO radicals, reducing protein oxidation. None of the BH improved plasma stress response. By reducing catalase levels without increasing LPO, the RNF treatment appears to adjust European seabass' antioxidant defences, indicating its potential to supply exogenous antioxidants to combat oxidative stress induced by ROS. However, this impact was not sufficient to lower LPO levels compared to a control plant-based diet. The tested diets seemed to affect the fish oxidative stress response in the liver, possibly due to the presence of bioactive peptides, which aided in the non-enzymatic modulation of stress response, as observed by the total antioxidant capacity values in the liver.

1. Introduction

The practice of farming marine carnivorous fish used to depend on the inclusion of high levels of marine-based ingredients, such as fishmeal (FM) and fish oil, in their diets. Nevertheless, the scarceness, increased prices and issues concerning the environmental unsustainability of these dietary components have motivated a search for alternative ingredient sources (Naylor et al., 2021). Regarding FM replacement, plant proteins

Abbreviations: ABTS•+, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; ACH50, alternative complement activity; AH-H, hydrolysate obtained by autohydrolysis; AH, diet with AH-H; BH, blood hydrolysates; BHT, butylated hydroxytoluene; BW, body weight; CAT, catalase; CC, carbonyl compounds; CDNB, 1-chloro-2,4-dinitrobenzene; CTRL, control diet; DM, dry matter; DPPH•, 1,1-diphenyl-2-picrylhydrazyl; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid, Ellman's reagent; DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylenediaminetetraacetic acid; EU, enzyme unit; FM, fishmeal; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; LPO, lipid peroxidation; MDA, malondialdehyde; NADPH, nicotinamide adenine dinucleotide phosphate; NEFA, non-esterified fatty acids; OD, optical density; ORAC, oxygen radical absorbance capacity; PUFAs, polyunsaturated fatty acids; RAS, recirculating aquaculture system; RMF-H, retentate from microfiltration; RMF, diet with RMF-H; RNF-H, retentate from nanofiltration; RNF, diet with RNF-H; ROS, reactive oxygen species; SOD, superoxide dismutase; TAC, total antioxidant capacity; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TCA, trichloroacetic acid; TG, total glutathione; TNB, 5-thio-2-nitrobenzoic acid.

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are the most used alternatives in aquafeeds (Abdel-Latif et al., 2022; Samuel-Fitwi et al., 2013). However, their use poses some challenges mainly related to food-feed competition, higher water and land requirements and deforestation (Aubin et al., 2019; Hua et al., 2019). Moreover, plant-based diets may also negatively impact fish growth, health and welfare, namely by compromising fish immunity and resistance to stress. Conde-Sieira et al. (2018) found some indications that vegetable diets may hinder fish welfare through alterations in the neurotransmission activity in the hypothalamus and telencephalon. Costa et al. (2020) found that a vegetable-based diet decreased plasmatic lysozyme in European seabass, which may compromise immunity. Torrecillas et al. (2017) found that reducing FM levels to 5% hindered gut associated lymphoid tissue capacity of response after a bacterial infection in European seabass. Finally, in the work of Yin et al. (2020), plant protein diets for hybrid groupers hindered antioxidant capacity and glycolipid metabolism, hampered intestinal development and decreased intestinal flora diversity, which the authors attribute to the presence of anti-nutritional factors. This is a major challenge to aquaculture intensification practices, including increased fish density, regular handling, transport and fishing, all of these being stress sources with negative repercussions on fish health (Sadoul et al., 2021). While stress itself may not be detrimental to fish health in the short term, if it becomes too intense or chronic, it may affect fish physiology at molecular and biochemical levels, impairing animal growth, immunity and survival (Barton, 2002; Sneddon et al., 2016).

Stress responses can be divided into primary, secondary or tertiary (Barton, 2002; Bonga, 1997; Sadoul et al., 2021). Primary responses are due to the activation of endocrine pathways, after recognition of a threat by the central nervous system. Secondary responses include respiratory and cardiovascular alterations due to hormonal action (Barton, 2002; Sadoul et al., 2021). Additionally, metabolic changes, such as increased levels of glucose and lactate and a decrease in tissue glycogen, are observed and the immune system can also be triggered. Finally, tertiary responses reflect the whole animal performance, with stress having ultimately negative impacts on fish growth, disease resistance, behaviour, and even survival (Ashley, 2007; Barton, 2002; Sadoul et al., 2021). Stress may overall lead to the accumulation of reactive oxygen species, ROS (Mohapatra et al., 2013), affecting the balance between ROS production and antioxidant scavenging capacity of tissues, which eventually damages biological molecules such as DNA, lipids and proteins, for example, through an increase in lipid peroxidation (LPO) or carbonyl compounds (CC) (Ko et al., 2014). To counteract the ROS, the organism activates antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) (Kurutas, 2016). However, exogenous antioxidants provided by aquafeeds may also help to maintain the ROS balance and mitigate oxidative damage.

Therefore, and considering the raising concerns regarding 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 fish farms (Ashley, 2007; Machado et al., 2019a). Thus, an emergent strategy for improving fish welfare is the supplementation of aquafeeds with bioactive compounds that provide physiological benefits beyond their pure nutritional value, which have particular relevance when used with plant-based diets (Encarnação, 2016; Olmos-Soto et al., 2015; Siddik et al., 2021). Among these bioactive compounds, protein hydrolysates have raised considerable interest from both the scientific community and the feed industry, as they can be obtained from by-products generated in food industries, which would make them attractive under a circular economy context (Faustino et al., 2019). The biological properties of hydrolysates have been increasingly demonstrated (Manzoor et al., 2022; Okoye et al., 2022; Siddik et al., 2021). These properties are highly dependent on the protein source and on the applied hydrolysis method. For instance, the inclusion of some anchovy hydrolysates in low FM diets for European seabass (*Dicentrarchus labrax*) led to improved lysozyme activity, when compared to a non-supplemented diet, but the usage of the same hydrolysate source with a different enzymatic processing did not lead to any changes in the same parameters (Costa et al., 2020). In juvenile red seabream (*Pagrus major*), Bui et al. (2014) demonstrated that 4.8% of a shrimp hydrolysate powder added to a fishmeal diet (47%) significantly increased plasma immunoglobulin, antiprotease and SOD activities. Additionally, in Japanese seabass (*Lateolabrax japonicus*) and yellow croaker (*Pseudosciaena crocea* R.), lysozyme activity and serum complement were enhanced by inclusion of fish protein hydrolysates (Liang et al., 2006; Tang et al., 2008). Inclusion of a tuna hydrolysate in a diet for barramundi (*Lates calcarifer*), at 10%, in diet with fermented poultry by-product meal led to increased activity of serum GPx (Siddik et al., 2020). Moreover, some hydrolysates have also been shown to improve stress response in mice (Chataigner et al., 2021; Dinel et al., 2021).

Previous work from our group demonstrated that a 3% swine blood hydrolysates (BH; obtained from a by-product of a pig slaughterhouse) inclusion in a plant-based diet led to an eight-fold reduction in European seabass mortality caused by *Tenacibaculum maritimum* infection (Resende et al., 2022), but the physiological mechanisms affected by such bioactive hydrolysates were not explored. Interest in blood hydrolysates arises from the fact that swine blood is an abundant by-product of meat production. Despite the absence of official reports of its global production, an estimation of 859 million L of swine blood can be produced yearly in the European Union (European Commission, 2021; Resende et al., 2022; Toldrà et al., 2019). Usually, it is processed into low-cost blood meal, but it could be valorised through hydrolysis yielding a beneficial mixture rich in bioactive peptides (Bah et al., 2013). In the present study, we propose to further evaluate the ability of BH included in low FM diets to modulate European seabass oxidative stress after a multifactorial acute stress challenge (air exposure followed by transfer to new tanks). This acute stress was chosen as being representative of frequent handling procedures that take place in fish farms, and to which fish response needs improving, through further dietary interventions (Machado et al., 2019a). In particular, some plasmatic metabolites and immune markers, hepatic oxidative stress markers and regulating enzymes, and muscle antioxidant potential were assessed.

2. Materials and methods

2.1. Ethical issues

The animal study protocol was approved by the Ethics Committee of CIIMAR for Managing Animal Welfare (ORBEA-CIIMAR_18_2017), in compliance with the Directive 2010/63/EU (European Union, 2010) and the Portuguese Decree_Law n° 113/2013 on "The protection of animals used for scientific purposes". The present study was performed by accredited scientists in laboratory animal science by the Portuguese Veterinary Authority (1005/92, DGV-Portugal, following FELASA category C recommendations).

2.2. Blood hydrolysates and experimental diets

Hydrolysis of swine blood was performed as described by Resende et al. (2022) and Araújo-Rodrigues et al. (2022). Two different processes were considered: enzymatic hydrolysis and autohydrolysis (the latter yielding the AH-H fraction). The hydrolysate mixture obtained enzymatically was further fractionated with a microfiltration (500 kDa cut-off), with the retentate from the microfiltration being termed RMF-H, while the filtrate was subjected to a nanofiltration (3 kDa cut-off). The retentate from this nanofiltration was called RNF-H. Peptide molecular weight (MW) profile of the hydrolysates is available on Table 1.

Four isonitrogenous (54% protein in dry matter, DM), isolipidic (16% DM) and isoenergetic (22 kJ g^{-1}) diets were produced: a plant-based diet with 12.5% FM was used as a control (control, CTRL) to which 3% of each BH was added, resulting in three further experimental

Table 1
Peptide molecular weight (MW) profile of the blood hydrolysates.

	Ingredients		
	RMF-H	RNF-H	AH-H
Peptide Profile (mAU)			
> 43 kDa	n.d.	n.d.	238137
29–43 kDa	765	32143	138862
13.7–29 kDa	1513	14786	80132
1.2–13.7	48017	373302	349651
< 1.2 kDa	7656	79596	131995

Source:Adapted from Resende et al. (2022).

diets (RMF, RNF, AH). Diets were formulated in compliance with European seabass nutritional requirements (National Research Council, 2011) and were extruded (2 mm) by SPAROS, Lda (Portugal). Table 2 describes the ingredients and proximate composition of diets.

2.3. Experimental design

European seabass juveniles were acquired from a commercial fish farm (Acuinuga, S.L., Spain) and transported to the Fish Culture Experimental Unit of CIIMAR, where the experiment was performed. After an acclimation period (15 days), 12 groups of 71 fish (weight of 12.3 ± 1.4 g, density of 3.5 kg m^{-3}), were randomly distributed by fiberglass tanks (250 L) within a recirculating aquaculture system (RAS). Nitrogenous compounds (NH_4^+ ≤0.05 mg L^{-1}; NO_2^- ≤0.5 mg L^{-1}; NO_3^- ≤5 mg L^{-1}), salinity (35 ± 1‰), temperature (20 ± 1 °C), dissolved oxygen (>90% saturation) and pH (7.5 ≤ pH ≤ 8.5) were monitored and kept at ideal levels for European seabass (Kır et al., 2019). Photoperiod was a cycle of 12 h light/12 h dark. Each tank received filtered saltwater at a flow rate of 16 L min^{-1}. Diets were randomly assigned to triplicate tanks and fish were fed 3 times daily until apparent visual satiety, for 74 days.

At the end of the trial, fish were fasted for 24 h. Afterwards, 3 fish per tank (9 fish per treatment) were either immediately sampled (non-stressed) or air-exposed for 1 min, placed in 50 L aerated tanks with clean saltwater and sampled (stressed) after a 6-hour recovery period, based on previous literature reports (Machado et al., 2019a; Zheng et al., 2017).

Immediately before sampling, all fish were sacrificed with anaesthetic overdose (0.5 mL L^{-1} of 2-Phenoxyethanol) and individually weighed and measured. Blood was taken from the caudal vein using heparinized syringes and centrifuged at 5000 g for 10 min at 4 °C, for plasma collection. Samples of liver and muscle were also collected and immediately frozen in liquid nitrogen. Plasma and tissue samples were kept at − 80 °C until analysis. The remaining fish were also lightly anesthetized with 2-Phenoxyethanol (60 μL L^{-1}), and individual weight and length were recorded.

Table 2
Ingredients, proximate composition and mineral composition of the diets used in the trial.

	Diets			
	CTRL	RMF	RNF	AH
Ingredients (%)				
Fishmeal (FM)[1]	12.50	12.50	12.50	12.50
Soy protein concentrate[2]	25.00	25.00	25.00	25.00
Wheat gluten[3]	13.50	10.00	10.10	10.20
Corn gluten[4]	15.00	15.00	15.00	15.00
Soybean meal 48[5]	10.00	10.00	10.00	10.00
Wheat meal[6]	7.24	7.44	7.34	7.34
Fish oil[7]	13.40	13.70	13.70	13.60
Vit & Min Premix[8]	0.50	0.50	0.50	0.50
DCP[9]	2.80	2.80	2.80	2.80
L-Tryptophan	0.06	0.06	0.06	0.06
RMF-H[10]	0	3.00	0	0
RNF-H[11]	0	0	3.00	0
AH-H[12]	0	0	0	3.00
Proximate composition (%DM)				
DM	96.24	94.63	97.00	93.96
Ash	7.93	7.73	7.87	8.04
Crude protein	54.24	54.72	54.57	54.63
Crude fat	15.93	15.79	16.21	16.03
Energy (kJ g^{-1})	22.37	22.44	22.55	22.69

CTRL, negative control; RMF, RNF, AH – diets supplemented with the respective hydrolysates. 1FM: 71% crude protein, 11% crude fat, EXALMAR, Peru; 2Soy protein concentrate: 65% protein, 0,7% lipids, ADM, Animal NutritionTM, The Netherlands; 3Wheat gluten: 90.1% DM, 83.8% protein, 1.6% lipids (as DM basis); 4Corn gluten feed: 61% crude protein, 6% crude fat, COPAM, Portugal; 5Dehulled solvent extracted soybean meal: 47.7% crude protein, 2.2% crude fat, CAR-GILL, Spain; 6Wheat meal: 10.2% protein, 1.2% lipids, Casa Lanchinha Lda., Portugal; 7Sardine oil, Sopropêche, France; 8Vitamins (IU or mg/kg diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20,000 IU; DL-cholecalciferol, 2000 IU; thiamine, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 1000 mg; inositol, 500 mg; biotin, 3 mg; calcium pantothenate, 100 mg; choline chloride, 1000 mg, betaine, 500 mg. Minerals (g or mg/kg diet): cobalt carbonate, 0.65 mg; copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate,7.5 mg; sodium chloride, 400 mg; calcium carbonate, 1.86 g; excipient wheat middlings. INVIVO 1%, Premix for marine fish, PREMIX Lda, Portugal; 9Di-calcium phosphate; 10Blood hydrolysate: retentate from microfiltration after enzymatic hydrolysis; 11Blood hydrolysate: retentate from nanofiltration after enzymatic hydrolysis; 12Blood hydrolysate: produced by auto-hydrolysis.
Source:Adapted from Resende et al. (2022).

2.4. Plasma analysis

All plasma analysis were determined in triplicate on a microplate spectrophotometer (BioTek Synergy HT, Vermont, USA).

2.4.1. Humoral immune parameters

Lysozyme activity (μg mL^{-1} of plasma) was quantified with a turbidimetric assay adapted to microtiter, as described by Hutchinson and Manning (1996) and Costa et al. (2020). A calibration curve with serially diluted, lyophilized hen egg white lysozyme (Sigma) was used.

Total peroxidase activity was measured following the procedure described by Quade and Roth (1997), by defining one unit of peroxidase as the amount which causes an absorbance change of one OD (EU mL^{-1} of plasma).

Alternative complement pathway activity (ACH50) analysis was based on the lysis of rabbit blood cells (Probiológica, Portugal), as described by Sunyer and Tort (1995). ACH50 units were set as the concentration of plasma that caused a cell lysis of 50%.

2.4.2. Metabolites in plasma

Plasma glucose, cholesterol, lactate and non-esterified fatty acids (NEFA) were determined enzymatically using commercial kits (Spinreact, Barcelona, Spain, for glucose, lactate and cholesterol; Wako Chemicals, Neuss, Germany, for fatty acids), adapted to a microplate format (Velasco et al., 2021).

2.5. Liver oxidative stress analysis

Liver samples were homogenized with phosphate buffer (0.1 M, pH 7.4), in a ratio of 1:10 (w/v). To 300 μL of liver homogenate, 5 μL of butylated hydroxytoluene (BHT, 4%, in methanol) were added, after which aliquots for LPO and CC were made and stored at − 80 °C. The remaining homogenate was centrifuged at 10 000 g at 4 °C for 20 min, after which the supernatant was extracted and stored at − 80 °C for antioxidant enzymes' analysis. Before freezing, the protein content of both homogenate and supernatant was measured as described by Bradford (1976) and applied to normalize antioxidant enzymes' activities.

2.5.1. Oxidative stress and antioxidant biomarkers

LPO was assessed in the liver homogenate through the quantification of thiobarbituric acid reactive substances (TBARS), in accordance with Bird and Draper (1984). LPO values were expressed as nmol TBARS/g fresh tissue. Regarding protein oxidation, this was assessed in the liver homogenate through the quantification of carbonyl compounds, using the Protein Carbonyl Content Assay Kit (Sigma-Aldrich MAK094–1KT), following the manufacturer's instructions. Values were expressed as nmol carbonyls per mg protein. The concentration of total antioxidants in liver samples was determined with the Total Antioxidant Capacity (TAC) Assay Kit (Sigma-Aldrich MAK187) and expressed as nmol of Trolox equivalents per g tissue.

Total glutathione (TG) was assessed via the formation of 5-thio-2-nitrobenzoic acid (TNB), at 412 nm, as detailed in Baker et al. (1990) and the results were expressed as nmol conjugated TNB formed per min per mg of protein.

2.5.2. Antioxidant enzyme activities

The activities of CAT, GPx, GR, GST and SOD were assessed in the supernatant, in triplicate using protocols adapted for microplate. CAT activity was evaluated as described by Claiborne (1985), with H_2O_2 30% as substrate. CAT activity was expressed in μmol consumed H_2O_2 per min per mg of protein. GPx activity was estimated considering NADPH oxidation at 340 nm, as stated by Mohandas et al. (1984). GPx activity was expressed as nmol NADPH oxidized per min per mg of protein. To assess GR activity, the method of Cribb et al. (1989) was employed and results were expressed as nmol oxidized NADPH min^{-1} mg $protein^{-1}$ ($\varepsilon = 6.22 \times 10^3\ M^{-1}cm^{-1}$).

The total GST activity was determined as described by Habig et al. (1974). Results were expressed in nmol CDNB conjugate formed per min per mg protein. Finally, SOD activity was measured using a SOD Determination Kit (Sigma-Aldrich 19160–1KT-F). Results were expressed as % of inhibition of the formation of WST-1 formazan per mg protein.

2.6. Muscle antioxidant potential

Muscle antioxidant potential was evaluated as described in Valente et al. (2015). Briefly, the muscle was hydrolyzed by pepsin under acidic conditions, and this reaction was inactivated by boiling at 100 °C. After centrifugation, the supernatant was recovered and analyzed for its antioxidant activity through the 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) and oxygen radical absorbance capacity (ORAC) tests (as it did not present significant 1,1-diphenyl-2-picrylhydrazyl, $DPPH^{\bullet}$, inhibition; data not shown), as described in Ribeiro et al. (2020). A calibration curve was developed with Trolox standards, and the results are expressed as nmol Trolox equivalents per mg muscle.

2.7. Statistical analysis

Data were tested for normality and homogeneity of variances, considering the Kolmogorov-Smirnov and Levene's tests, respectively, and, if necessary, appropriately transformed. A two-way ANOVA was used to analyse data, considering the diet and stress as fixed factors, with the Statistica v13.5 (TIBCO Software Inc., Palo Alto, CA, USA) software. If significant effects were found, a pairwise multiple comparison test (Tukey HSD) was performed. The minimum level of significance was set at $P < 0.05$ for all analyses. Furthermore, Pearson's correlations were evaluated for the data and considered a two-tailed analysis of 0.05 and 0.01.

3. Results

3.1. Growth performance

A more detailed analysis of the impact of the hydrolysates on growth performances is available on Resende et al. (2022). Briefly, RNF did not differ from CTRL in the final fish weight (47.01 ± 7.30 vs. 48.93 ± 8.21, respectively; $P = 0.065$). AH displayed a significantly lower final body weight (46.62 ± 7.76; $P < 0.001$) than CTRL, but it was similar to RNF. Finally, RMF was the worst performing diet (33.37 ± 5.27, $P < 0.001$).

3.2. Plasma metabolites and innate immune markers analysis

Regarding the analysed plasma metabolites (Table 3), cortisol and lactate levels were significantly increased in stressed fish, thus validating our experimental design. However, no significant differences were found among diets. Glucose, cholesterol and triglycerides were unaffected by either diet or stress. NEFA was not affected by stress alone, but non-stressed fish fed AH displayed the highest levels.

Protein levels were not only reduced in stressed fish, but were also affected by diet since the RMF group displayed significantly lower protein levels than all other dietary treatments.

In terms of the analysed innate immune markers (Table 4), ACH50 and peroxidase were unaffected by either stress or dietary treatments. Lysozyme was not affected by the stress, but it was significantly reduced by the RMF diet.

3.3. Liver oxidative stress

LPO was significantly increased post-stress (Fig. 1). Moreover, while the non-stressed RMF-fed fish displayed the lowest LPO values, the stressed fish fed the same diet displayed the highest values. Prior to the stress, the AH diet displayed higher LPO than the RMF and RNF. However, after the stress, all diets were statistically similar. No differences among dietary treatments were found for carbonyl compounds levels, but stressed fish had lower levels compared to non-stressed. Regarding liver TAC, while both stressed and non-stressed fish fed CTRL display statistically similar levels, a significant decrease is seen when comparing stressed to non-stressed fish fed the BH diets.

Significant differences among stressed and non-stressed fish were observed in CAT and GPx, but dietary treatments did not have a significant impact. GR was highest for stressed AH-fed fish. The stressed AH group also showed an increase in GST activity, being significantly higher than the stressed control fish. RMF led to TG and SOD values that were significantly higher than all other diets.

3.4. Muscle antioxidant potential

Fig. 2 depicts the results from muscle antioxidant potential. When evaluated through ABTS, a significant increase was seen for stressed fish. However, this was not observed in the ORAC analysis, as no differences regarding stress or dietary treatments were found.

3.5. Pearson correlations

Some of the Pearson's are described in Table 5 and a full table is provided as supplementary material. Liver LPO displayed strong correlations with SOD activity and lactate, and weaker, but significant correlations with TAC, CAT and plasma cortisol. Furthermore, carbonyl levels were strongly but negatively correlated with TAC, CAT, cortisol and lactate. SOD was correlated with cortisol and CAT. Cortisol and lactate were also strongly correlated.

4. Discussion

As fish welfare becomes a major concern in aquaculture production, aquafeed development evolves to not only provide the basic nutrients, but to also enhance fish response to stressful situations. In this sense, we have hypothesized that functional feeds, low FM aquafeeds supplemented with blood hydrolysates, could improve the animals' response to stress conditions faced in farms.

Table 3
Plasma metabolite levels of fish prior to (non-stressed) or after (stressed) an air exposure.

	Diets								Two-way ANOVA (*P*-value)		
	Non-Stressed				Stressed						
	CTRL	RMF	RNF	AH	CTRL	RMF	RNF	AH	Diet	Stress	Diet × Stress
Cortisol (ng mL^{-1})	618 ± 101	589 ± 91	529 ± 47	652 ± 68	1014 ± 92	875 ± 91	859 ± 115	790 ± 74	0.420	< 0.001	0.419
Glucose (mmol L^{-1})	7.82 ± 0.89	7.17 ± 0.24	7.33 ± 0.36	9.58 ± 0.45	8.08 ± 0.48	8.57 ± 0.87	8.36 ± 0.39	8.84 ± 0.22	0.040 *	0.206	0.229
Lactate (mmol L^{-1})	3.69 ± 0.60	2.74 ± 0.33	2.75 ± 0.20	2.83 ± 0.15	3.84 ± 0.41	4.17 ± 0.42	4.01 ± 0.37	4.37 ± 0.35	0.797	< 0.001	0.873
Triglycerides (mmol L^{-1})	5.46 ± 1.08	5.49 ± 1.00	6.30 ± 1.11	5.86 ± 0.90	4.94 ± 0.63	4.46 ± 0.96	6.73 ± 1.43	4.90 ± 0.37	0.503	0.532	0.948
Cholesterol (mmol L^{-1})	7.92 ± 1.70	6.65 ± 1.04	8.66 ± 1.06	8.11 ± 1.15	7.27 ± 0.68	5.87 ± 1.26	7.01 ± 0.62	8.00 ± 0.66	0.380	0.310	0.919
NEFA (mmol L^{-1})	0.12 ± 0.01^{B}	0.14 ± 0.01^{B}	0.13 ± 0.01^{B}	0.20 ± 0.02 ^{A}	0.15 ± 0.01^{AB}	0.11 ± 0.01^{B}	0.12 ± 0.01^{B}	0.16 ± 0.01^{AB}	< 0.001	0.126	0.014
Protein (g dL^{-1})	4.23 ± 0.31^{a}	3.63 ± 0.26^{b}	4.12 ± 0.21^{a}	4.23 ± 0.19^{a}	3.94 ± 0.14^{a}	2.68 ± 0.27^{b}	3.72 ± 0.21^{a}	4.03 ± 0.20^{a}	< 0.001	0.007	0.348

Values are presented as mean ± SE. Different superscript lowercase letters denote significant differences among diets ($P < 0.05$), while different superscript uppercase letters indicate significant differences for Diet × Stress. * No significant differences were found after the post-hoc test.

Table 4
Humoral immune parameters of fish before (non-stressed) or after (stressed) air exposure.

	Diets								Two-way ANOVA (*P*-value)		
	Non-Stressed				Stressed						
	CTRL	RMF	RNF	AH	CTRL	RMF	RNF	AH	Diet	Stress	Diet × Stress
Lysozyme (µg mL^{-1})	15.74 ± 1.03^{a}	12.25 ± 0.76^{b}	14.41 ± 1.03^{a}	16.66 ± 0.68^{a}	15.99 ± 0.75^{a}	9.21 ± 0.49^{b}	15.49 ± 1.29^{a}	15.85 ± 1.19^{a}	<0.001	0.353	0.171
Peroxidase (EU mL^{-1})	33.8 ± 4.6	74.9 ± 18.0	33.8 ± 5.6	39.4 ± 5.1	62.0 ± 11.5	43.7 ± 7.5	55.0 ± 12.5	30.5 ± 3.4	0.146	0.749	0.034*
ACH50 (units mL^{-1})	167.6 ± 18.7	136.8 ± 31.1	111.6 ± 19.5	135.6 ± 30.6	127.1 ± 8.7	130.1 ± 7.8	126.1 ± 10.3	163.1 ± 9.4	0.217	0.901	0.279

Values are presented as mean ± SE. Different superscript lowercase letters denote significant differences among diets ($P<0.05$), while different superscript uppercase letters indicate significant differences for Diet × Stress.
* No significant differences were found after the post-hoc test.

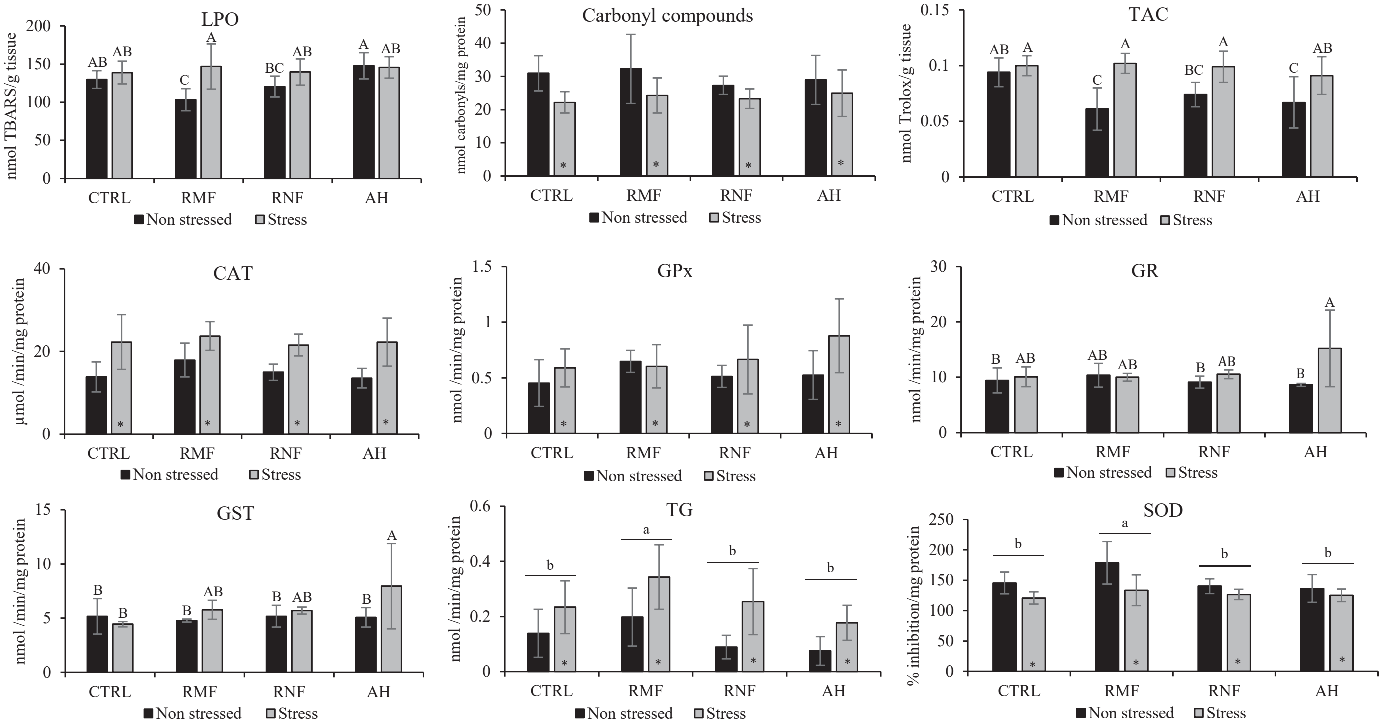

Fig. 1. Liver oxidative status and antioxidant enzymes' activity. LPO – lipid peroxidation; TAC – total non-enzymatic antioxidant activity; CAT – catalase; GPx – glutathione peroxidase; GR – glutathione reductase; GST – glutathione S-transferase; TG – total glutathione; SOD – superoxide dismutase. Values are presented as mean ± SE (n = 9). Different superscript lowercase letters denote significant differences among diets ($P < 0.05$), while different superscript uppercase letters indicate significant differences for Diet × Stress ($P < 0.05$). * indicates significant differences between stressed and non-stressed fish ($P < 0.05$).

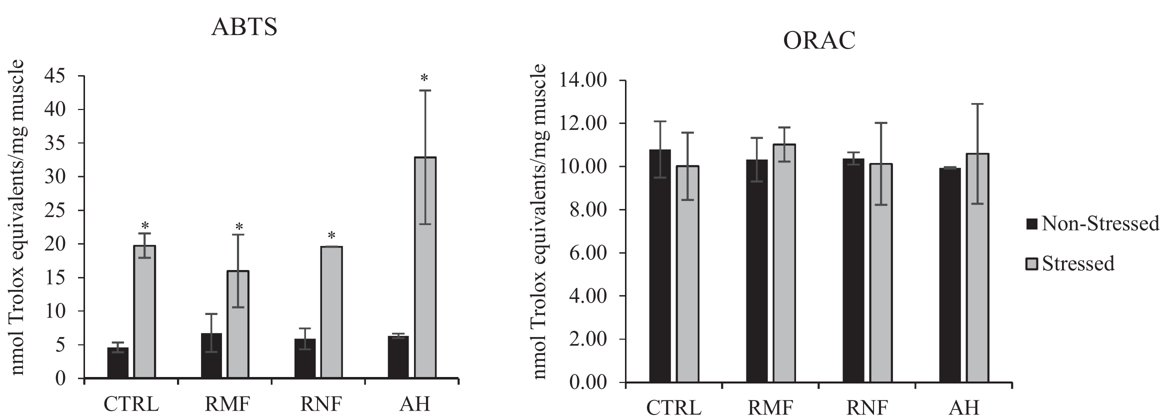

Fig. 2. Antioxidant potential of the muscle of fish fed the experimental diets, evaluated through 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) and oxygen radical absorbance capacity (ORAC) tests. Values are presented as mean ± SE (n = 9). Different superscript lowercase letters denote significant differences among diets ($P < 0.05$), while different superscript uppercase letters indicate significant differences for Diet × Stress ($P < 0.05$). Asterisk (*) indicates significant differences between stressed and non-stressed fish ($P < 0.05$).

The evaluated stress challenge was an acute stress, with total duration of 1 min, followed by sampling after a recovery period of 6 h. The significantly increased responses on cortisol, lactate and liver lipid peroxidation in stressed fish validate the employed protocol, since these are typical indicators of acute stress (Barton, 2002; Cerqueira et al., 2021; Ciji and Akhtar, 2021). Cortisol is released by interrenal cells as a response to the secretion of adrenocorticotropin by the anterior pituitary, which had been previously stimulated by the corticotropin-releasing hormone produced by the hypothalamus. Thus, cortisol response is delayed a few minutes from stress recognition by the central nervous system (Barton, 2002; Ciji and Akhtar, 2021). While this is a common stress biomarker, it has a high degree of biological variability, even in individuals subjected to the same experimental conditions (Ciji and Akhtar, 2021; de Magalhães et al., 2020). Indeed, high standard error values were found in cortisol measurements, and while the impact of stress was visible, other more subtle differences caused by diets may be disguised by this high variability.

Cortisol promotes both glycogenolysis and gluconeogenesis, which may increase glucose levels, to yield the fish with the necessary energy to counteract the stressor (Martinez-Porchas et al., 2009). Fanouraki et al. (2011) provided useful information on the general patterns of the response of plasma cortisol, lactate, and glucose concentrations in seabass after acute stress. They observed that both glucose (12 mmol L^{-1}) and lactate (9 mmol L^{-1}) levels were considerably higher than in the present study (8.5 mmol L^{-1} and 4 mmol L^{-1}, respectively), even 8 h post-stress. Yet, they found that plasma glucose after 4 h dropped to

Table 5
Pearson correlations between some evaluated parameters.

		LPO	Carbonyls	TAC	GPx	SOD	CAT	Cortisol	Lactate
LPO	Pearson correlation	1	-0.260*	0.295*	0.047	-0.503**	0.250*	0.284*	0.305**
	Sig (2-tailed)		0.027	0.034	0.697	< 0.001	0.034	0.016	0.009
Carbonyls	Pearson correlation		1	-0.327**	-0.035	0.179	-0.363**	-0.342**	-0.320**
	Sig (2-tailed)			0.005	0.771	0.133	0.002	0.003	0.006
TAC	Pearson correlation			1	-0.028	-0.560**	-0.364**	0.338**	0.413**
	Sig (2-tailed)				0.817	< 0.001	0.002	0.004	< 0.001
GPx	Pearson correlation				1	-0.045	0.192	0.157	0.334**
	Sig (2-tailed)					0.707	0.106	0.189	0.004
SOD	Pearson correlation					1	-0.241*	-0.286*	-0.223
	Sig (2-tailed)						0.042	0.015	0.060
CAT	Pearson correlation						1	0.355**	0.431**
	Sig (2-tailed)							0.002	< 0.001
Cortisol	Pearson correlation							1	0.468**
	Sig (2-tailed)								< 0.001
Lactate	Pearson correlation								1
	Sig (2-tailed)								

* significant at $p < 0.05$
** significant at $p < 0.01$

basal levels for most of the species investigated. In our work, despite an absence of statistical differences, glucose levels 6 h post-stress are slightly higher for all groups except for fish fed AH. All these observations could provide evidence and support for the idea that glucose levels after 6 h may already have returned to basal levels. Rotllant and Tort (1997) reported that after an acute stress (net handling for 8 min), glucose levels of red porgy *Pagrus pagru* were elevated after a two-hour period, but not after 24 h. Davis and McEntire (2009) have described that glucose levels of sunshine bass (a hybrid *Morone chrysops X Morone saxatilis*) and white bass (*Morone chrysops*) only slightly rise after a stress (decrease in water volume in the tank) and are returned to normal after 6 h. Another hypothesis, stated by Martinez-Porchas et al. (2009) is that, while cortisol does increase circulating glucose levels, the stress may also increase the rate at which fish consume the energetic substrates. Moreover, in a stressful event, the animal's oxygen demand may increase, leading to cell hypoxia and anaerobic metabolism of glucose. This explains the increase in circulating lactate levels after the challenge and the absence of significant differences in glucose levels post-stress (Mirzargar et al., 2022).

Overall, the hydrolysates had a reduced impact on plasma stress indicators. Similarly, another work with swine blood by-products at 5% in diets for *Argyrosomus regius* also did not find any differences in glucose, lactate, protein or cortisol, although these biomarkers were evaluated on skin mucus rather than plasma, and without subjecting the animals to any challenge (Fernández-Alacid et al., 2021). It could be possible that further increased inclusion levels could have better outcomes, since the average value of cortisol was lower for BH diets, albeit not significantly.

Plasma protein levels can be an indicator of the nutritional status of fish (Pelusio et al., 2022). In this work, they decreased after the stress challenge, in accordance with previous literature reports (Di Marco et al., 2008; Fernández-Alacid et al., 2019; Mommsen et al., 1999; Samaras et al., 2023). This decrease can be attributed to proteolysis derived from cortisol action (Di Marco et al., 2008; Mommsen et al., 1999; Pelusio et al., 2022). This proteolytic action has been claimed to be evident in white muscle (Mommsen et al., 1999) and can also explain the results from the ABTS tests, as an increased antioxidant potential was observed in muscle, probably derived from peptides released from this proteolysis. However, the ORAC test did not support these results. This is not unheard of, as the $ABTS^{\bullet+}$ is sterically-hindered stable, compared to the tested radical in the ORAC test, leading to the observed difference (Schaich et al., 2015). ORAC uses a biologically pertinent radical source (H_2O_2), meaning its results should be closer to an in vivo approach (Thaipong et al., 2006); yet this relates more to a possible benefit of fillets for consumers than to inferences regarding proteolysis of muscle. Additionally, the RMF diet led to lower plasmatic protein levels, which suggests an overall poorer nutritional status and possible higher protein oxidation. This is in agreement with its general worse growth performance (in terms of final weight), when compared to the remaining diets. Nevertheless, further analysis regarding amino acid and protein turnover should be performed to validate this evidence.

High values of NEFA in the plasma can be attributed to a mobilization of lipid reserves and their oxidation, caused by a higher metabolic demand (Di Marco et al., 2008). The AH diet led to the highest NEFA values, and also displayed high levels of liver LPO, particularly in non-stressed fish, which could be an indicator of impairment of lipid metabolism in fish fed this diet. In a previous work with these blood hydrolysates, a reduction of lipid digestibility was observed for this diet (Resende et al., 2022), which supports this hypothesis. However, despite alterations in NEFA, triglycerides and cholesterol were not affected, a similar situation to that reported by Di Marco et al. (2008). This could indicate that free fatty acids may derive from mesenteric fat or hepatic tissue, rather than from blood triglycerides, which suggests the activation of transcription factors that control metabolic pathways in a tissue-specific manner, regulating nutrient transport and modulating levels of plasma NEFA, triglycerides and cholesterol (Shearer et al., 2012).

Humoral immune parameters were not affected by stress in this report, despite the frequent association between both chronical and acute oxidative stress and impairments in the immune system (Dawood et al., 2022; Paray et al., 2021). It is, however, possible that acute stressors may not affect seriously the immune system, as other responses of the organism are occurring (Tort, 2011). This is in agreement with Machado et al. (2019a), who also found that a similar stress for European seabass did not affect some immune markers such as peroxidase and lysozyme. Additionally, the BH did not affect significantly the innate immune parameters in the plasma, compared to the CTRL. Diet RMF led to lower plasmatic lysozyme levels, possibly suggesting a poorer capacity to respond in the event of an infection. This hydrolysate possessed the lowest abundance of smaller sized peptides, which have been associated with beneficial effects on immunity (Resende et al., 2022; Siddik et al., 2021), and that could be the reason behind those values. In any case, the values observed for plasmatic lysozyme (Machado et al., 2019b), peroxidase (Campos et al., 2017) and ACH50 (Azeredo et al., 2017) are in agreement with previous reports for this species.

Other authors have found that marine hydrolysates in low FM diets increased the non-specific immunity biomarkers to values equal to or above to those induced by a non-supplemented FM-based diet (Costa et al., 2020; Gisbert et al., 2018). Yet, Leduc et al. (2018) claimed that a shrimp hydrolysate had stronger immunostimulant properties than a tilapia hydrolysate when supplemented at equal levels in European seabass diets. Thus, the raw material used to produce hydrolysates plays an important role in determining the outcomes, and this could be the reason behind the differences in our results, along with variations in inclusion levels of the hydrolysates. Other residual compounds, such as vitamins or minerals, eventually present in the hydrolysates, may also play an important role. In addition, it is suggested that, in future experiments, other immune markers, namely expression of immunoglobulins, antiproteases, or even inflammation markers, are assessed to obtain a clearer picture of the potential of hydrolysates to modulate immune responses.

Another concern regarding the impacts of stress and impaired fish welfare is related to the production of ROS, which then tend to accumulate in the liver (Awasthi et al., 2018). Indeed, liver LPO levels were increased after stress, similarly to previous literature reports on this species (Silva-Brito et al., 2019). Liver LPO pre-stress was lowest for the RMF diet, but this diet displayed the highest increase after the challenge. This could be partially due to the decrease in SOD levels after stress. SOD is an enzyme that catalyses the conversion of the superoxide radical into molecular oxygen and hydrogen peroxide, being an important antioxidant defence (Birben et al., 2012). Indeed, we have found a negative correlation between liver SOD activity and liver LPO ($p < 0.001$), as previously demonstrated by other authors in studies correlating higher SOD activity with lower LPO levels (Abdel-Tawwab et al., 2021; Passos et al., 2021).

Liver CAT activity was higher for stressed fish than for non-stressed ones and has a positive correlation with LPO. This suggests that once stressed, the increase in lipid peroxidation stimulates the activity of CAT, which aids in antioxidant defence by breaking down hydrogen peroxide (a pro-oxidant) into water and molecular oxygen (Moutinho et al., 2021). The results here presented for CAT are within the range described for this species (Islam et al., 2020).

GPx acts in a similar way to CAT, also converting hydrogen peroxide into water, and the range of its activity is within the range reported for this species (Lobo et al., 2018). Nevertheless, GPx is not significantly correlated with CAT in this work. GPx activity was higher for stressed fish in all diets apart from RMF, where it was decreased. This may also partially explain the increase of LPO in stressed fish fed this diet, due to the impairment of antioxidant enzymatic defences.

However, while the activity of SOD was significantly lower in all diets after the stress challenge, only the RMF diet displayed a significant increase in LPO (considering the two-way ANOVA results). Exogenous antioxidants present in the diet, such as bioactive peptides, can enhance non-enzymatic antioxidant response and minimize the need for enzymatic activity when facing oxidative stress (Batista et al., 2020; Moutinho et al., 2021; Pereira et al., 2022). The TAC values support this hypothesis, as a mobilization of non-enzymatic antioxidants seems evident in BH-diets post-stress. Furthermore, TAC has a negative correlation with SOD activity, which suggests that if non-enzymatic antioxidants are mobilized, the need for antioxidant enzymes decreases. It is possible that some of the bioactive peptides present in the BH could have an affinity for the superoxide radical in the liver of the stressed fish, minimizing the need for the SOD activity. However, further assessment of the mechanisms of action of such peptides are still needed to clarify this matter.

The AH diet required higher activities of GPx, GR, and GST, which can suggest that this diet cannot modulate antioxidant defences, leading for higher needs of the enzymes to maintain homeostasis. This occurred despite the presence of low-sized (<13.7 kDa) peptides in this hydrolysate. A low bioavailability of such peptides could be the reason behind its inefficacy. Indeed, a previous work with this diet resulted in worse protein digestibility and higher faecal nitrogen losses (Resende et al., 2022), which would affect not only protein metabolism related to growth, but also reduce the availability of bioactive peptides. The RNF, which also displays small sized peptides, led to statistically similar GR and GST activities before and after the stress. As such, the bioactive peptides present in this potentially functional feed may be more available than those present in AH.

In oxidative stress events, ROS may directly or indirectly introduce carbonyl moieties at amino acid side chains. Higher amounts of carbonyl groups have been correlated with protein damage resulting from oxidative stress (Almroth et al., 2005). However, in this work, liver carbonyls are significantly decreased in stressed fish compared to non-stressed. Two reasons, previously stated in the literature, may aid explain this issue. Firstly, mildly oxidated proteins are more susceptible to proteolytic degradation, as a defence mechanism to prevent the spreading of oxidative damage (Passi et al., 2004). Moreover, the increase of lipid peroxidation products has been reported to act as an inhibitor for protein carbonyl formation (Almroth et al., 2005). Indeed, a negative correlation between carbonyls and LPO was observed. Therefore, both an increase and a decrease in carbonyls can be indicators of oxidative stress (Almroth et al., 2005); the RNF and AH diets, which led to the lowest difference between the non-stressed and stressed counterparts, could possibly be adequate to prevent an elevated impact of oxidative stress on proteins.

5. Conclusions

The tested diets seemed to affect the fish oxidative stress response in the liver. This could be due to their bioactive peptides, which aided in the non-enzymatic modulation of stress response. This seems to be confirmed by the TAC values in the liver. However, this impact was not sufficient to lower LPO levels compared to a control plant-based diet. In addition, plasmatic cortisol response was not affected by dietary treatments. Therefore, and while bioactive hydrolysates remain an option for improvement of farmed fish stress response, further research is needed. This includes optimizing inclusion levels, assessing other timepoints after an acute stress and verifying the impact of BH on chronical stress.

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CRediT authorship contribution statement

Daniela Resende: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ricardo Pereira:** Data curation, Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **David Domínguez:** Investigation, Writing – review & editing. **Miguel Pereira:** Formal analysis, Investigation, Writing – review & editing. **Carlos Pereira:** Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. **Manuela Pintado:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Luísa M.P. Valente:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Cristina Velasco:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2023.101600.

References

Abdel-Latif, H.M.R., Abdel-Daim, M.M., Shukry, M., Nowosad, J., Kucharczyk, D., 2022. Benefits and applications of *Moringa oleifera* as a plant protein source in aquafeed: a review. Aquaculture 547, 737369. https://doi.org/10.1016/j.aquaculture.2021.737369.

Abdel-Tawwab, M., Khalil, R.H., Diab, A.M., Khallaf, M.A., Abdel-Razek, N., Abdel-Latif, H.M.R., Khalifa, E., 2021. Dietary garlic and chitosan enhanced the antioxidant capacity, immunity, and modulated the transcription of HSP70 and Cytokine genes in Zearalenone-intoxicated European seabass. Fish. Shellfish Immunol. 113, 35–41. https://doi.org/10.1016/j.fsi.2021.03.012.

Almroth, B.C., Sturve, J., Berglund, Å., Förlin, L., 2005. Oxidative damage in eelpout (*Zoarces viviparus*), measured as protein carbonyls and TBARS, as biomarkers. Aquat. Toxicol. 73, 171–180. https://doi.org/10.1016/j.aquatox.2005.03.007.

Araújo-Rodrigues, H., Coscueta, E.R., Pereira, M.F., Cunha, S.A., Almeida, A., Rosa, A., Martins, R., Pereira, C.D., Pintado, M.E., 2022. Membrane fractionation of *Cynara cardunculus* swine blood hydrolysate: ingredients of high nutritional and nutraceutical value. Food Res. Int. 158, 111549 https://doi.org/10.1016/j.foodres.2022.111549.

Ashley, P.J., 2007. Fish welfare: current issues in aquaculture. Appl. Anim. Behav. Sci. 104, 199–235. https://doi.org/10.1016/j.applanim.2006.09.001.

Aubin, J., Callier, M., Rey-Valette, H., Mathé, S., Wilfart, A., Legendre, M., Slembrouck, J., Caruso, D., Chia, E., Masson, G., Blancheton, J.P., Ediwarman, Haryadi, J., Prihadi, T.H., de Matos Casaca, J., Tamassia, S.T.J., Tocqueville, A., Fontaine, P., 2019. Implementing ecological intensification in fish farming: definition and principles from contrasting experiences. Rev. Aquac. 11, 149–167. https://doi.org/10.1111/raq.12231.

Awasthi, Y., Ratn, A., Prasad, R., Kumar, M., Trivedi, S.P., 2018. An in vivo analysis of Cr^{6+} induced biochemical, genotoxicological and transcriptional profiling of genes related to oxidative stress, DNA damage and apoptosis in liver of fish, *Channa punctatus* (Bloch, 1793). Aquat. Toxicol. 200, 158–167. https://doi.org/10.1016/j.aquatox.2018.05.001.

Azeredo, R., Machado, M., Kreuz, E., Wuertz, S., Oliva-Teles, A., Enes, P., Costas, B., 2017. The European seabass (*Dicentrarchus labrax*) innate immunity and gut health are modulated by dietary plant-protein inclusion and prebiotic supplementation. Fish. Shellfish Immunol. 60, 78–87. https://doi.org/10.1016/j.fsi.2016.11.019.

Bah, C.S.F., Bekhit, A.E.-D.A., Carne, A., McConnell, M.A., 2013. Slaughterhouse blood: an emerging source of bioactive compounds. Compr. Rev. Food Sci. Food Saf. 12, 314–331. https://doi.org/10.1111/1541-4337.12013.

Baker, M.A., Cerniglia, G.J., Zaman, A., 1990. Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. Anal. Biochem. 190, 360–365. https://doi.org/10.1016/0003-2697(90)90208-Q.

Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42, 517–525. https://doi.org/10.1093/icb/42.3.517.

Batista, S., Pereira, R., Oliveira, B., Baião, L.F., Jessen, F., Tulli, F., Messina, M., Silva, J. L., Abreu, H., Valente, L.M.P., 2020. Exploring the potential of seaweed *Gracilaria gracilis* and microalga *Nannochloropsis oceanica*, single or blended, as natural dietary ingredients for European seabass *Dicentrarchus labrax*. J. Appl. Phycol. 32, 2041–2059. https://doi.org/10.1007/s10811-020-02118-z.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O., 2012. Oxidative stress and antioxidant defense. World Allergy Organ. J. 5, 9–19. https://doi.org/10.1097/WOX.0b013e3182439613.

Bird, R.P., Draper, H.H., 1984. Comparative studies on different methods of malonaldehyde determination. Methods Enzym. 105, 299–305. https://doi.org/10.1016/s0076-6879(84)05038-2.

Bonga, S.E.W., 1997. The stress response in fish. Physiol. Rev. 77, 591–625. https://doi.org/10.1152/physrev.1997.77.3.591.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

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 418–419, 11–16. https://doi.org/10.1016/j.aquaculture.2013.09.046.

Campos, I., Matos, E., Marques, A., Valente, L.M.P., 2017. Hydrolyzed feather meal as a partial fishmeal replacement in diets for European seabass (*Dicentrarchus labrax*) juveniles. Aquaculture 476, 152–159. https://doi.org/10.1016/j.aquaculture.2017.04.024.

Cerqueira, M., Millot, S., Silva, T., Félix, A.S., Castanheira, M.F., Rey, S., Mackenzie, S., Oliveira, G.A., Oliveira, C.C.V., Oliveira, R.F., 2021. Stressor controllability modulates the stress response in fish. BMC Neurosci. 22, 48. https://doi.org/10.1186/s12868-021-00653-0.

Chataigner, M., Mortessagne, P., Lucas, C., Pallet, V., Layé, S., Mehaignerie, A., Bouvret, E., Dinel, A.L., Joffre, C., 2021. Dietary fish hydrolysate supplementation containing n-3 LC-PUFAs and peptides prevents short-term memory and stress response deficits in aged mice. Brain Behav. Immun. 91, 716–730. https://doi.org/10.1016/j.bbi.2020.09.022.

Ciji, A., Akhtar, M.S., 2021. Stress management in aquaculture: a review of dietary interventions. Rev. Aquac. 13, 2190–2247. https://doi.org/10.1111/raq.12565.

Claiborne, A., 1985. Catalase activity. In: Greenwald, R. (Ed.), Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, FL, USA, pp. 283–284.

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

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.

Cribb, A.E., Leeder, J.S., Spielberg, S.P., 1989. Use of a microplate reader in an assay of glutathione reductase using 5,5′-dithiobis(2-nitrobenzoic acid). Anal. Biochem. 183, 195–196. https://doi.org/10.1016/0003-2697(89)90188-7.

Davis, K., McEntire, M., 2009. Comparison of the cortisol and glucose stress response to acute confinement among white bass, *Morone chrysops*, striped bass, *Morone saxatilis*, and sunshine bass, *Morone chrysops* X *Morone saxatilis*. J. World Aquac. Soc. 40, 567–572. https://doi.org/10.1111/j.1749-7345.2009.00275.x.

Dawood, M.A.O., Alkafafy, M., Sewilam, H., 2022. The antioxidant responses of gills, intestines and livers and blood immunity of common carp (*Cyprinus carpio*) exposed to salinity and temperature stressors. Fish. Physiol. Biochem. 48, 397–408. https://doi.org/10.1007/s10695-022-01052-w.

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, 319–328. https://doi.org/10.1016/j.aquaculture.2007.12.012.

Dinel, A.-L., Lucas, C., Le Faouder, J., Bouvret, E., Pallet, V., Layé, S., Joffre, C., 2021. Supplementation with low molecular weight peptides from fish protein hydrolysate reduces acute mild stress-induced corticosterone secretion and modulates stress responsive gene expression in mice. J. Funct. Foods 76, 104292. https://doi.org/10.1016/j.jff.2020.104292.

Encarnação, P., 2016. Functional feed additives in aquaculture feeds. In: Nates, S.F. (Ed.), Aquafeed Formulation. Academic Press, San Diego, CA, USA, pp. 217–237.

European Commission, 2021. Pigmeat statistics. Retrieved from https://ec.europa.eu/info/food-farming-fisheries/farming/facts-and-figures/markets/overviews/market-observatories/meat/pigmeat-statistics_en on 08-07-2021.

Fanouraki, E., Mylonas, C.C., Papandroulakis, N., Pavlidis, M., 2011. Species specificity in the magnitude and duration of the acute stress response in Mediterranean marine fish in culture. Gen. Comp. Endocrinol. 173, 313–322. https://doi.org/10.1016/j.ygcen.2011.06.004.

Faustino, M., Veiga, M., Sousa, P., Costa, E.M., Silva, S., Pintado, M., 2019. Agro-food byproducts as a new source of natural food additives. Molecules 24, 1056. https://doi.org/10.3390/molecules24061056.

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.

Fernández-Alacid, L., Firmino, J.P., Sanahuja, I., Madrid, C., Polo, J., de Borba, M.R., Balsalobre, C., Gisbert, E., Ibarz, A., 2021. Impact of dietary porcine blood by-products in meagre (*Argyrosomus regius*) physiology, evaluated by welfare biomarkers and the antibacterial properties of the skin mucus. Fish. Shellfish Immunol. 118, 241–250. https://doi.org/10.1016/j.fsi.2021.09.011.

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.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8.

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, 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, U. K. Fish. Shellfish Immunol. 6, 473–482. https://doi.org/10.1006/fsim.1996.0045.

Islam, M.J., Kunzmann, A., Thiele, R., Slater, M.J., 2020. Effects of extreme ambient temperature in European seabass, *Dicentrarchus labrax* acclimated at different salinities: growth performance, metabolic and molecular stress responses. Sci. Total Environ. 735, 139371 https://doi.org/10.1016/j.scitotenv.2020.139371.

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.

Ko, J.-Y., Kim, E.-A., Lee, J.-H., Kang, M.-C., Lee, J.-S., Kim, J.-S., Jung, W.-K., Jeon, Y.-J., 2014. Protective effect of aquacultured flounder fish-derived peptide against oxidative stress in zebrafish. Fish. Shellfish Immunol. 36, 320–323. https://doi.org/10.1016/j.fsi.2013.11.018.

Kurutas, E.B., 2016. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr. J. 15, 71. https://doi.org/10.1186/s12937-016-0186-5.

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., 2018. 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 Genom. 19, 396. 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). Aquac. Res. 37, 102–106. https://doi.org/10.1111/j.1365-2109.2005.01392.x.

Lobo, G., Pereira, L.F., Gonçalves, J.F.M., Peixoto, M.J., Ozório, R.O.A., 2018. Effect of dietary seaweed supplementation on growth performance, antioxidant and immune responses in European seabass (*Dicentrarchus labrax*) subjected to rearing temperature and salinity oscillations. Int. Aquat. Res. 10, 321–331. https://doi.org/10.1007/s40071-018-0208-3.

Machado, M., Castro, C., Oliva-Teles, A., Costas, B., 2019a. 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.

Machado, M., Azeredo, R., Domingues, A., Fernandez-Boo, S., Dias, J., Conceição, L.E.C., Costas, B., 2019b. Dietary tryptophan deficiency and its supplementation compromises inflammatory mechanisms and disease resistance in a teleost fish. Sci. Rep. 9, 7689. https://doi.org/10.1038/s41598-019-44205-3.

de Magalhães, C.S.F.R., Cerqueira, M.A.C., Schrama, D., Moreira, M.J.V., Boonanuntanasarn, S., Rodrigues, P.M.L., 2020. A Proteomics and other Omics approach in the context of farmed fish welfare and biomarker discovery. Rev. Aquac. 12, 122–144. https://doi.org/10.1111/raq.12308.

Manzoor, M., Singh, J., Gani, A., 2022. Exploration of bioactive peptides from various origin as promising nutraceutical treasures: *In vitro*, *in silico* and in vivo studies. Food Chem. 373, 131395 https://doi.org/10.1016/j.foodchem.2021.131395.

Martinez-Porchas, M., Martinez-Cordova, L.R., Ramos-Enriquez, R., 2009. Cortisol and glucose: reliable indicators of fish stress? Panam. J. Aquat. Sci. 4, 158–178.

Mirzargar, S.S., Taheri Mirghaed, A., Hoseini, S.M., Ghelichpour, M., shahbazi, M., Yousefi, M., 2022. Biochemical responses of common carp, *Cyprinus carpio*, to transportation in plastic bags using thymol as a sedative agent. Aquac. Res. 53, 191–198. https://doi.org/10.1111/are.15564.

Mohandas, J., Marshall, J.J., Duggin, G.G., Horvath, J.S., Tiller, D.J., 1984. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: possible implications in analgesic nephropathy. Biochem. Pharm. 33, 1801–1807. https://doi.org/10.1016/0006-2952(84)90353-8.

Mohapatra, S., Chakraborty, T., Kumar, V., DeBoeck, G., Mohanta, K.N., 2013. Aquaculture and stress management: a review of probiotic intervention. J. Anim. Physiol. Anim. Nutr. 97, 405–430. https://doi.org/10.1111/j.1439-0396.2012.01301.x.

Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish. Biol. Fish. 9, 211–268. https://doi.org/10.1023/a:1008924418720.

Moutinho, S., Pedrosa, R., Magalhães, R., Oliva-Teles, A., Parisi, G., Peres, H., 2021. Black soldier fly (*Hermetia illucens*) pre-pupae larvae meal in diets for European seabass (*Dicentrarchus labrax*) juveniles: effects on liver oxidative status and fillet quality traits during shelf-life. Aquaculture 533, 736080. https://doi.org/10.1016/j.aquaculture.2020.736080.

National Research Council, 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC.

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, 551–563. https://doi.org/10.1038/s41586-021-03308-6.

Okoye, C.O., Ezeorba, T.P.C., Okeke, E.S., Okagu, I.U., 2022. Recent findings on the isolation, identification and quantification of bioactive peptides. Appl. Food Res. 2, 100065 https://doi.org/10.1016/j.afres.2022.100065.

Olmos-Soto, J., Paniagua-Michel, J., López, L., Ochoa, L., 2015. In: Kim, S.-K. (Ed.), Functional feeds in aquaculture. Springer Handbook of Marine Biotechnology: Part J - Industrial Applications, Berlin, Germany, pp. 1303–1319.

Paray, B.A., El-Basuini, M.F., Alagawany, M., Albeshr, M.F., Farah, M.A., Dawood, M.A. O., 2021. *Yucca schidigera* usage for healthy aquatic animals: potential roles for sustainability. Animals 11, 93. https://doi.org/10.3390/ani11010093.

Passi, S., Ricci, R., Cataudella, S., Ferrante, I., De Simone, F., Rastrelli, L., 2004. Fatty acid pattern, oxidation product development, and antioxidant loss in muscle tissue of rainbow trout and *Dicentrarchus labrax* during growth. J. Agric. Food Chem. 52, 2587–2592. https://doi.org/10.1021/jf030559t.

Passos, R., Correia, A.P., Pires, D., Pires, P., Ferreira, I., Simões, M., do Carmo, B., Santos, P., Pombo, A., Afonso, C., Baptista, T., 2021. Potential use of macroalgae *Gracilaria gracilis* in diets for European seabass (*Dicentrarchus labrax*): Health benefits from a sustainable source. Fish. Shellfish Immunol. 119, 105–113. https://doi.org/10.1016/j.fsi.2021.09.033.

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. Front. Mar. Sci. 8, 779053 https://doi.org/10.3389/fmars.2021.779053.

Pereira, R., Costa, M., Velasco, C., Cunha, L.M., Lima, R.C., Baião, L.F., Batista, S., Marques, A., Sá, T., Campos, D.A., Pereira, M., Jesus, D., Fernández-Boo, S., Costas, B., Pintado, M., Valente, L.M.P., 2022. Comparative analysis between synthetic vitamin E and natural antioxidant sources from tomato, carrot and coriander in diets for market-sized *Dicentrarchus labrax*. Antioxidants 11, 636. https://doi.org/10.3390/antiox11040636.

Quade, M.J., Roth, J.A., 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Vet. Immunol. Immunopathol. 58, 239–248. https://doi.org/10.1016/s0165-2427(97)00048-2.

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.

Ribeiro, T.B., Oliveira, A., Campos, D., Nunes, J., Vicente, A.A., Pintado, M., 2020. Simulated digestion of an olive pomace water-soluble ingredient: relationship between the bioaccessibility of compounds and their potential health benefits. Food Funct. 11, 2238–2254. https://doi.org/10.1039/C9FO03000J.

Rotllant, J., Tort, L., 1997. Cortisol and glucose responses after acute stress by net handling in the sparid red porgy previously subjected to crowding stress. J. Fish. Biol. 51, 21–28. https://doi.org/10.1111/j.1095-8649.1997.tb02510.x.

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 (737071–737072), 737017. https://doi.org/10.1016/j.aquaculture.2021.737072.

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.

Samuel-Fitwi, B., Meyer, S., Reckmann, K., Schroeder, J.P., Schulz, C., 2013. Aspiring for environmentally conscious aquafeed: comparative LCA of aquafeed manufacturing using different protein sources. J. Clean. Prod. 52, 225–233. https://doi.org/10.1016/j.jclepro.2013.02.031.

Schaich, K.M., Tian, X., Xie, J., 2015. Reprint of "Hurdles and pitfalls in measuring antioxidant efficacy: a critical evaluation of ABTS, DPPH, and ORAC assays". J. Funct. Foods 18, 782–796. https://doi.org/10.1016/j.jff.2015.05.024.

Shearer, G.C., Savinova, O.V., Harris, W.S., 2012. Fish oil — how does it reduce plasma triglycerides? Biochim. Biophys. Acta - Mol. Cell. Biol. Lipids 1821, 843–851. https://doi.org/10.1016/j.bbalip.2011.10.011.

Siddik, M.A.B., Chaklader, M.R., Foysal, M.J., Howieson, J., Fotedar, R., Gupta, S.K., 2020. Influence of fish protein hydrolysate produced from industrial residues on antioxidant activity, cytokine expression and gut microbial communities in juvenile barramundi Lates calcarifer. Fish. Shellfish Immunol. 97, 465–473. https://doi.org/10.1016/j.fsi.2019.12.057.

Siddik, M.A.B., Howieson, J., Fotedar, R., Partridge, G.J., 2021. Enzymatic fish protein hydrolysates in finfish aquaculture: a review. Rev. Aquac. 13, 406–430. https://doi.org/10.1111/raq.12481.

Silva-Brito, F., Timóteo, F., Esteves, Â., Peixoto, M.J., Ozorio, R., Magnoni, L., 2019. Impact of the replacement of dietary fish oil by animal fats and environmental salinity on the metabolic response of European Seabass (*Dicentrarchus labrax*). Comp. Biochem. Physiol. B 233, 46–59. https://doi.org/10.1016/j.cbpb.2019.04.004.

Sneddon, L.U., Wolfenden, D.C.C., Thomson, J.S., 2016. Stress Management and Welfare. In: Schreck, C.B., Tort, L., Farrell, A.P., Brauner, C.J. (Eds.), Fish Physiology. Academic Press, Cambridge, MA, USA, pp. 463–539.

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

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.). J. Zhejiang Univ. Sci. B. 9, 684–690. https://doi.org/10.1631/jzus.B0820088.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Hawkins Byrne, D., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compos. Anal. 19, 669–675. https://doi.org/10.1016/j.jfca.2006.01.003.

Toldrà, M., Lynch, S.A., Couture, R., Álvarez, C., 2019. Chapter 5 - Blood Proteins as Functional Ingredients. In: Galanakis, C.M. (Ed.), Sustainable Meat Production and Processing. Academic Press, London, pp. 85–101.

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. Shellfish Immunol. 67, 302–311. https://doi.org/10.1016/j.fsi.2017.06.022.

Tort, L., 2011. Stress and immune modulation in fish. Dev. Comp. Immunol. 35, 1366–1375. https://doi.org/10.1016/j.dci.2011.07.002.

Valente, L.M.P., Rema, P., Ferraro, V., Pintado, M., Sousa-Pinto, I., Cunha, L.M., Oliveira, M.B., Araújo, M., 2015. Iodine enrichment of rainbow trout flesh by dietary supplementation with the red seaweed *Gracilaria vermiculophylla*. Aquaculture 446, 132–139. https://doi.org/10.1016/j.aquaculture.2015.05.004.

Velasco, C., Conde-Sieira, M., Comesaña, S., Chivite, M., Míguez, J.M., Soengas, J.L., 2021. Role of the G protein-coupled receptors GPR84 and GPR119 in the central regulation of food intake in rainbow trout. J. Exp. Biol. (jeb242360), 224. https://doi.org/10.1242/jeb.242360.

Yin, B., Liu, H., Tan, B., Dong, X., Chi, S., Yang, Q., Zhang, S., 2020. Preliminary study of mechanisms of intestinal inflammation induced by plant proteins in juvenile hybrid groupers (♀*Epinephelus fuscoguttatus*×♂*E. lanceolatu*). Fish. Shellfish Immunol. 106, 341–356. https://doi.org/10.1016/j.fsi.2020.07.026.

Zheng, J.-L., Zeng, L., Xu, M.-Y., Shen, B., Wu, C.-W., 2017. Different effects of low- and high-dose waterborne zinc on Zn accumulation, ROS levels, oxidative damage and antioxidant responses in the liver of large yellow croaker *Pseudosciaena crocea*. Fish. Physiol. Biochem. 43, 153–163. https://doi.org/10.1007/s10695-016-0275-6.

The supplementary material (*Appendix A. Supplementary material*) relative to this journal article is available at: https://www.sciencedirect.com/science/article/pii/S2352513423001394?via%3Dihub#sec0125.

4. INCORPORATION OF AROMAS FROM THE CANNING INDUSTRY

4.1. Production of aroma-rich extracts from sardine cooking wastewaters: Exploring their potential for modulating feed intake in European seabass

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ORIGINAL PAPER

Production of Aroma-Rich Extracts from Sardine Cooking Wastewaters: Exploring Their Potential for Modulating Feed Intake in European Seabass

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Abstract
Managing the canning industry's nutrient-rich and odorous liquid waste is a hurdle. Concurrently, the growing use of vegetable ingredients in aquafeeds diminishes palatability and feed consumption in carnivorous fish. Thus, we hypothesized that aromas could be extracted from cooking wastewaters at canning factories and added to plant-based diets to stimulate intake in European seabass. Sardine cooking wastewaters were collected and tested directly (CW-A) or after vacuum distillation (VD-A) or liquid/liquid extraction with soybean oil (LLE-A). Despite losses in aldehydes and short-chain alcohols, both processes were effective in removing off-flavours. VD-A displayed a higher concentration of most aromas compared to LLE-A. Extracts were included at 2 µg g^{-1} of 1-penten-3-ol, the most abundant compound in all extracts, in diets (CW, VD, LLE). A non-supplemented diet was used as control. Each diet was assigned to six groups of juvenile fish, fed a single meal until apparent satiation. Our emphasis was on this initial feeding to comprehend the hedonic control of feed intake, minimizing habituation effects and the impact of the long-term metabolic requirements. Feed intake was highest for the control group. No differences on plasma metabolites were observed, suggesting feed intake was primarily regulated by hedonic rather than homeostatic mechanisms. Moreover, the lower intake in the supplemented diets was partially associated with a lower expression of orexigenic (intake-promoting) neuropeptides and higher expression of anorexigenic (intake-reducing) neuropeptides in the brain, despite the lack of significant diet-related differences. Overall, this study presents a novel approach to valorise cooking wastewater from the canning industry, since cooking wastewaters extracts rich in aromas were successfully produced, however, in the tested concentration, had no positive impact on the short-term feed intake response of European seabass.

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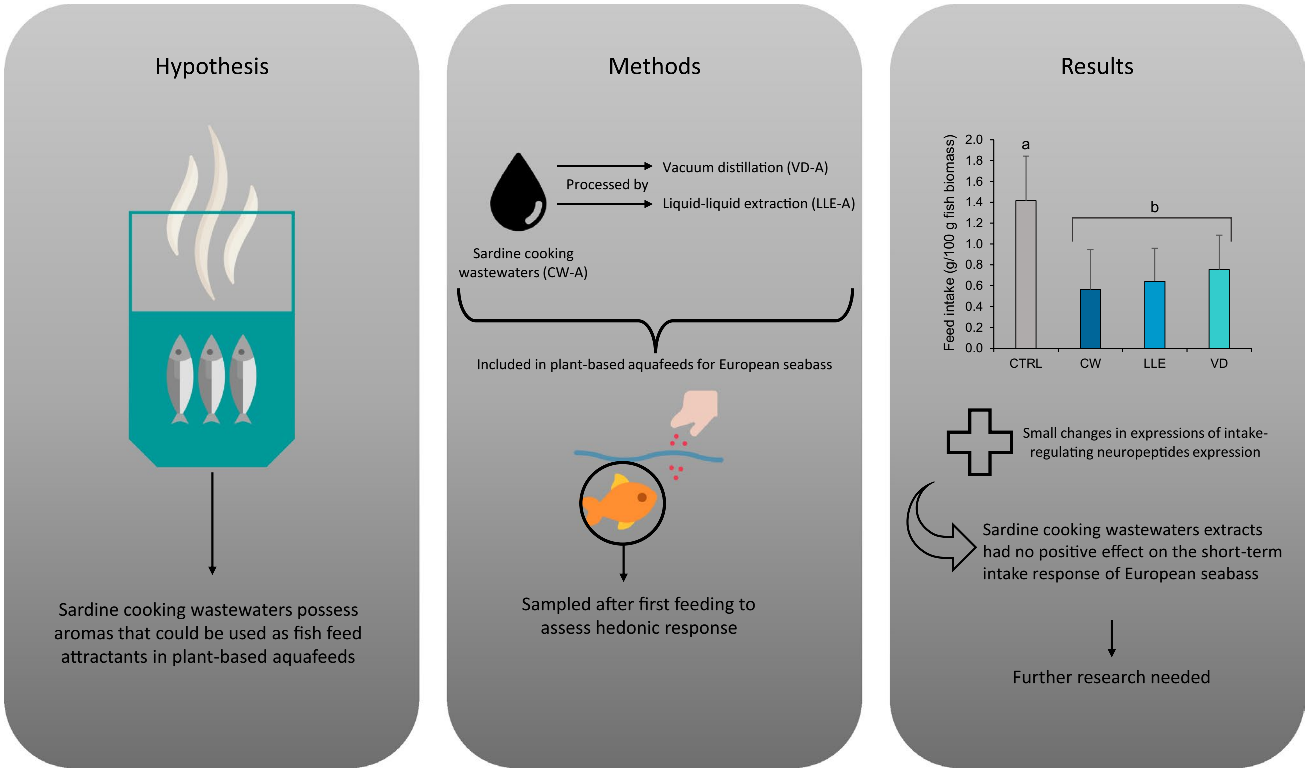
Graphical Abstract



Keywords Appetite stimulants · Palatability · Zero waste · Circular economy

Introduction

Currently, one of the key problems worldwide is waste management and this challenge is further aggravated by the growing complexity and quantities of industrial wastes produced all over the world. In this sense, reuse and valorisation of wastes and effluents is particularly significative.

The canning industry represents 11% of the worldwide fish market. In the particular case of Portugal, over 56 thousand tonnes of canned fish were produced in 2021, of which 20% corresponded to sardine products [1]. It is worth noting that for the production of each tonne of canned fish, up to 9 m^3 of liquid waste are generated [2]. This liquid waste is particularly complicated to process due to its high levels of salts, organic matter and odorous volatile compounds, including alcohols, aldehydes and ketones, which are released during the cooking process. Often, wastewaters are processed in conventional treatment plants with flotation devices, although other methods have been suggested, including evaporation processes [3], biotransformation [4, 5], and membrane filtration [4, 6]. Additionally, these novel methods may allow the recovery of concentrated fatty acids, proteins/peptides or even aromas, which could have further applications in other industries and contribute to the valorisation of this waste.

In parallel, the aquaculture sector faces several challenges regarding the sustainability of aquafeed production. Marine-based aquafeed ingredients, such as fishmeal (FM) and fish oil (FO), due to their scarceness, increasing prices and unsustainability, are being replaced by other sources, such as plant proteins and oils, which could minimize such issues [7]. Nevertheless, such replacement can be hazardous, due to the inclusion of antinutritional factors, inadequate amino acid or fatty acid profile and poorer nutrient digestibility [8, 9]. In addition, a major detrimental effect of these ingredients is the reduced palatability, and therefore reduced feed intake. Geurden et al. [10] noticed a reduction in feed intake when switching from a marine-based diet to a plant-based one, during the first week of feeding of rainbow trout (*Oncorhynchus mykiss*). Liang et al. [11] observed anorexia in *Lateolabrax japonicas*, when dietary FM was totally substituted by a plant protein blend, during the first 2 weeks of feeding. Similarly, Sabioni et al. [12] reported that the dietary inclusion of a soy protein concentrate above 50% diminished feed intake in dorado (*Salminus brasiliensis*) by modulating certain appetite-regulating hormones.

Furthermore, Torstensen et al. [13] showed a reduced intake in Atlantic salmon (*Salmo salar*) smolts fed a diet with 80% of plant proteins and 70% vegetable oils, compared to a marine-based aquafeed, after three months of feeding. Even a mid-term reduction in feed intake followed by habituation to high-vegetable diets, where significant differences in feed intake patterns disappear, may affect overall growth performance of the fish, and cause large production losses [13], as this reduction may have impacted physiological and endocrine functions.

Moreover, feed can account for up to 40–60% of the production costs in fish farms [14], being a leading cause of waste production, because of leftover uneaten feed or dietary digestibility issues [15, 16]. Thus, the feed industry has been seeking novel strategies to stimulate feed intake, without compromising feed efficiency.

Feed intake modulation relies on the interaction of both homeostatic and hedonic signals, with the first relating to the animal's needs to fulfil energy requirements, and the latter resulting from pleasant sensations derived from feed [17]. While this is a complex regulating mechanism that involves several central and peripheral tissues and sensory organs, an integration can be found at the central nervous system level, mostly at the fish's hypothalamus and telencephalon [18, 19]. In this sense, feed attractants can directly interact with taste or olfactive receptors, promoting a hedonic response that results in increased feed intake. Interaction of food odours and olfactive receptors produces olfactory signals which are then transmitted to the brain, where they are integrated in the feed intake regulation network [20]. Although significant progress has been made, there remains ongoing exploration into the intricate mechanisms modulating feed intake regulation in fish. Ultimately, integration of homeostatic and hedonic signals affects the abundance of specific neuropeptides which may in turn affect feed intake, either suppressing it (anorexigenic) or stimulating it (orexigenic). These neuropeptides include the anorexigens cocaine and amphetamine-related transcript (CART) and pro-opiomelanocortin (POMC) and the orexigens agouti-related peptide (AgRP) and neuropeptide Y (NPY) [21, 22]. While neuropeptide expression in the hypothalamus has been closely linked with the homeostatic regulation, their role in the telencephalon might relate more to a hedonic regulation, although this is still unclear. Other mechanisms, such as the opioid system and endocannabinoid system, are also believed to play a role in the hedonic control of feed intake [23, 24], but their interaction with the expression of the aforementioned neuropeptides is still unclear. Over time fish can display habituation to diets, even if they are not particularly pleasing, due to nutrient necessities. Hence, evaluating feed intake and its regulation after short feeding trials can be an opportunity to screen for potential feed attractants and predict how they would affect fish feed intake [17, 22, 25], particularly regarding the impact of these attractants in the hedonic response, since the impact of metabolic needs in the first meal would be minimized.

Therefore, one way to overcome the issue of plant-based aquafeeds reduce palatability is the use of feed attractants [26]. Furthermore, fish display a very high sensibility to tastants and odorants, when compared to most mammals [20, 27]. In this regard, a number of feed attractants have been described in the literature, mostly including amino acids [28] and peptides/hydrolysates [29]. Nonetheless, compounds such as alcohols, aldehydes and organic acids, which have been reported to stimulate food intake in mammals [30, 31] are still widely unexplored in fish species. Moreover, feed attractants must be not only effective as such, but also economically and environmentally sustainable, and this could be achieved by valorising agri-food by-products, simultaneously contributing to circular economy policies [32]. As such, we have hypothesized that cooking wastewaters from the canning industry, characterized by a "fish smell", might be harnessed as a source of valuable aroma compounds for utilization as feed attractants. Thus, the goals of this work encompassed the extraction of aromas from sardine cooking wastewaters and the assessment of their potential as short-term feed intake stimulants in plant-based diets for European seabass.

Materials and Methods

Production of Aroma Concentrate Extracts

Effluent Collection and Reverse Osmosis

Sardine cooking wastewater from a local canning industry (A Poveira S.A., Laúndos, Portugal) was obtained after steaming sardines at 100 °C during 7 min. An antioxidant extract (acorn extract; 1% v/v) was added at the outlet of the cooking chambers to prevent lipid oxidation and aroma deterioration. This cooking wastewater aroma with the antioxidant, CW-A, was stored at −20 °C until further processed. A 100 L sample of CW-A was centrifuged (industrial WestFalia type ADB skimming centrifuge) for 30 min, resulting in one aqueous and one organic fraction. A reverse osmosis process with a TW30LE—2540 membrane (AMFOR, Inc, USA), with an area of 2.6 m^2, was then applied to the aqueous fraction to achieve a fraction richer in aromas.

Vacuum Distillation

This fraction (600 mL) was evaporated using a rotary evaporator (R-210, BÜCHI Labortechnik AG, Switzerland), under a feed temperature of 30 °C, and further distilled using a vacuum pressure of 25 mbar and a condensation temperature of 0 °C. The final vacuum distillate extracted aroma, VD-A, was stored at −20 °C until further use.

Liquid–Liquid Extraction

The ratio *oil:feed* for liquid–liquid extraction with soy oil was set based on the ratio *final condensate:feed* achieved for the vacuum distillation process. Thus, extraction was performed at 30 °C, with agitation for 15 min at 240 rpm, using 10% soy oil, followed by centrifugation at 17,000 × *g* for 20 min. The liquid–liquid extracted aroma was named LLE-A and kept at −20 °C until use.

Diet Preparation

For the feeding trial, four isonitrogeneous (52% protein in dry matter, DM) and isolipidic (18% DM) diets were formulated, complying with European seabass nutritional requirements (National Research Council, 2011). The control (CTRL) was a practical plant-based diet, with 12.5% FM

Table 1 Identified aroma compounds in sardine cooking wastewaters and aromatic concentrates

Aroma compounds	CW-A		VD-A		LLE-A	
	Area	Conc.	Area	Conc.	Area	Conc.
Aldehydes						
Pentanal	342,274,014				242,047,008	
Hexanal	91,764,044				333,718,748	
Heptanal	55,695,101	0.008	3,057,639	0.050		
2-Hexenal, (E)-	224,708,703					
2-Heptenal, (Z)-					52,659,617	
Nonanal	62,709,909		91,162,319		11,237,254	
2-Octenal, (E)-	93,582,059		25,531,262		7,592,788	
2,4-Heptadienal	537,113,248	0.060	31,038,604	0.030	1,474,782	0.002
2,6-Nonadienal, (E,Z)-	128,500,271	0.060	122,048,941	0.059		
Alcohols						
1-Penten-3-ol	621,242,979	2.080	211,923,385	0.247	39,359,694	0.055
2-Penten-1-ol, (E)-	28,574,975		27,468,182		0	
2-Penten-1-ol, (Z)-	88,113,043.67	0.160	59,594,494	0.103	0	
1-Hexanol			212,159,530		71,875,385	
2-Hexen-1-ol, (E)-	38,769,652.33		23,485,942		9,079,827	
1-Octen-3-ol	155,667,784	0.005	252,085,522	0.010	16,925,971	0.002
1-Heptanol			143,858,319		5,919,625	
(5Z)-Octa-1,5-dien-3-ol	212,332,868		183,893,384		4,327,839	
1-Octanol			2,710,154,269		42,707,256	
1-Decanol					49,946,449	
Ketones						
2-Nonanone	72,661,377	0.003	104,099,006	0.005		
3,5-Octadien-2-one			81,267,303		16,549,597	
(3E,5E)-3,5-octadien-2-one	73,914,891		459,011,183		3,991,195	
2-Undecanone	31,415,463		191,942,580			
Acids						
Pentanoic acid					12,101,580	
Hexanoic acid					6,570,463	
Alkanes						
Pentadecane	66,233,128					
Heptadecane	152,304,028					
Sulphur compounds						
trans-2-(2-Pentenyl)furan	437,467,260					

Concentrations are shown in ppm

CW-A cooking wastewaters with antioxidant extract, *VD-A* extract from vacuum distillation, *LLE-A* extract from liquid–liquid extractions and 4% FO. This was chosen due to the growing tendency of the use of plant-based ingredients and their known impact in feed intake [10–12]. Considering 1-penten-3-ol was the most abundant compound in the extracts (Table 1) and is also responsible for the flavour of fresh marine products [6, 33], aroma inclusion levels were set as to incorporate the same amount of this alcohol in all supplemented diets. The three experimental diets (CW, LLE, VD) were developed through the addition of each aromatic extract to the soybean oil fraction of the CTRL diet. All diets were extruded, as described in Resende et al. [34], and the oil fraction was further added by coating, in order to avoid extract degradation caused by extrusion temperatures during pellet manufacturing. Experimental diets were produced by SPAROS Lda. and stored at 4 °C until use. Table 2 describes diets ingredients and proximate composition.

Feeding Trial

Juvenile European seabass were acquired from a commercial fish farm (Atlantik Fish Lda., Portugal) and transported to the Fish Culture Experimental Unit of CIIMAR (Matosinhos, Portugal). Fish were kept in quarantine, in a 2000 L tank included in a recirculating saltwater system (RAS) for 2 weeks and, during this period, they were fed a commercial diet (Aquasoja, Sorgal S.A.; 50% crude protein, 20% crude fat, as DM basis) once daily. Nitrogenous compounds ($NH_4^+ \leq 0.05$ mg L^{-1}; $NO_2^- \leq 0.5$ mg L^{-1}; $NO_3^- \leq 5$ mg L^{-1}) salinity (35‰), temperature (21 ± 1 °C), dissolved oxygen (>90% saturation) and pH (7.5 ≤ pH ≤ 8.5) were regularly monitored and maintained at levels recommended for this species [35]. Photoperiod was a cycle of 12 h light/12 h dark.

After the quarantine period, fish were individually weighed (96.0 ± 13.8 g) and measured (total length, 20.7 ± 1.0 cm). Then, 24 homogeneous groups of 4 fish were randomly distributed by 50 L fiberglass tanks within a RAS (2.5 L min^{-1} flow rate). The remaining environmental conditions were the same as the quarantine.

After a 15-day acclimatisation period to the tanks, each diet was randomly assigned to six groups of fish that were fed until apparent satiation with the experimental diets. Sampling was performed 2 and 6 h after this single meal, where two fish were collected from three different tanks per diet (six fish/treatment) for each time point, to avoid handling stress at the second sampling point. Sampling times were selected based on previous similar studies [36, 37].

For the sampling, fish were collected and sacrificed by anaesthetic overdose (2-phenoxyethanol, Sigma-Aldrich, MO, USA). Fish were individually weighed (g) and measured (total length, cm). Blood was taken from the caudal vein using heparinized syringes and centrifuged (5000 × *g*, 10 min) for plasma collection, which was stored at −80 °C until analysis. Hypothalamus and telencephalon were collected, flash-frozen in dry ice and stored at −80 °C until mRNA extraction and quantification.

Table 2 Formulation and chemical composition of diets used in the feeding trial

	Diets			
	CTRL	CW	LLE	VD
Ingredients (%)				
Fishmeal[a]	12.5	12.5	12.5	12.5
Soy protein concentrate[b]	25.0	25.0	25.0	25.0
Wheat gluten[c]	15.0	15.0	15.0	15.0
Corn gluten meal[d]	10.0	10.0	10.0	10.0
Soybean meal[e]	11.0	11.0	11.0	11.0
Wheat meal[f]	9.1	9.1	9.1	9.1
Vitamin and mineral premix[g]	0.5	0.5	0.5	0.5
DCP[h]	1.5	1.5	1.5	1.5
Fish oil[i]	4.0	4.0	4.0	4.0
Soybean oil[j]	11.4	11.4	7.7	11.4
CW (mL)[k]		12.0		
LLE[l]			3.7	
VC (mL)[m]				100.0
Chemical composition (%DM)				
Dry matter	95.24	94.53	95.08	94.53
Ash	6.52	6.51	6.57	6.51
Crude protein	52.19	52.28	52.15	52.28
Crude fat	18.12	17.87	17.58	17.87
Energy (kJ/g)	22.65	22.84	22.68	22.84
Phosphorus	0.85	0.86	0.87	0.86
1-Penten-3-ol (μg/g)	0.98	2.10	2.16	2.10

[a]Fishmeal NORVIK LT, Sopropêche, France (72% crude protein, 7% crude fat)

[b]Soy protein concentrate Soycomil®-P, ADM, Animal Nutrition™, Netherlands (65% protein, 0.7% lipids)

[c]Wheat gluten composition: DM: 901 g kg^{-1}; protein: 838 g kg^{-1}; lipids: 16 g kg^{-1}

[d]Corn gluten feed from COPAM, Portugal (61% crude protein, 6% crude fat)

[e]Dehulled solvent extracted soybean meal, CARGILL, Spain (48% crude protein, 2.2% crude fat)

[f]Wheat meal from Casa Lanchinha Lda., Portugal (10.2% protein, 1.2% lipids)

[g]INVIVO 1%, Premix for marine fish, PREMIX Lda., Portugal

[h]Di-calcium phosphate

[i]Sardine oil, Sopropêche, France

[j]Henry Lamotte Oils, GmbH, Germany

[k]Extract from sardine cooking wastewaters

[l]Extract from sardine cooking wastewaters processed through liquid/liquid extraction

[m]Extract from sardine cooking wastewaters processed through vacuum distillation

Chemical Analysis

Diets were ground and analysed according to AOAC methods, as described in Resende et al. [34]. In short, dry matter (105 °C, 24 h); ash (combustion at 550 °C for 6 h, muffle furnace, Nabertherm L9/11/B170, Bremen, Germany); crude fat (petroleum ether extraction; Soxtec™ 2055, FOSS, Höganäs, Sweden); crude protein (N × 6.25; Leco nitrogen analyser FP-528, Leco Corporation, St. Joseph, USA); gross energy (adiabatic bomb calorimeter; Werke C2000, IKA, Staufen, Germany); and phosphorus (acid digestion of ashes, then phosphates quantification with ammonium molybdate via absorbance reading at 820 nm) were assessed in duplicate. Moreover, for identification of the present compounds and quantification of 1-penten-3-ol in aroma concentrate extracts and diets, SPME/GC–MS (solid phase microextraction followed by gas chromatography–mass spectrometry) was applied, according to the protocol reported in Pereira et al. [6]. A Shimadzu gas chromatograph (GCMS-QP2010) equipped with a WAX column (30 m × 0.25 mm i.d. × 0.25 µm) was used. The carrier gas was ultrapure helium, at 1 mL min^{-1}. The oven temperature program was as follows: 60 °C (4 min), followed by a gradual increase of 2 °C min^{-1} up to 180 °C. Injector temperature was 200 °C (limited by the desorption temperature expressed by the SPME fibre manufacturer). Detector temperature was fixed at 220 °C and ionization source at 200 °C. The ionization mode was electron impact with electron energy of 70 eV. A volume of 6 mL was then extracted by CAR/PDMS fibre during 15 min to 60 °C with and without stirring. The time of analyte desorption from the SPME fibre was set at 10 min. The injection was performed in the spitless mode for 2 min. After this period, the split ratio was set at 1:20 until the end of the chromatographic run. The internal standard was 2-nonanol. NaCl (2 g) was added to all analysed samples, in order to achieve a good extraction of the compounds.

Plasma Metabolites Quantification

Glucose, lactate, cholesterol and non-esterified fatty acids (NEFA) were examined enzymatically using commercial kits (Spinreact, Barcelona, Spain, for glucose, lactate and cholesterol; Wako Chemicals, Neuss, Germany, for fatty acids), adapted to microplate, as described in Velasco et al. [38]. All analysis were run in triplicate on a microplate spectrophotometer (BioTek Synergy HT, Vermont, USA).

mRNA Quantification of Neuropeptides Through RT-qPCR

Total RNA was extracted from whole hypothalamus or telencephalon using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations, with some modifications, followed by purification with a NZY Total RNA Isolation Kit (NZYTech, Lisbon, Portugal) as described by Ferreira et al. [39]. RNA quantity and purity was evaluated with a DeNovix DS-11FX spectrophotometer (Wilmington, DE, USA) and assessed based on the absorbance ratio 260:280 nm. Samples with a A_{260}/A_{280} ratio of 1.80–2.10 were considered for analysis. Then, 1.5 µg of RNA was reverse transcribed to cDNA with the NZY First-Strand cDNA Synthesis Kit (NZYTech, Lisbon, Portugal), following the manufacturer's instructions.

Expression of neuropeptide y (*npy*), agouti-related peptide (*agrp2*), cocaine and amphetamine-related transcript (*cartpt2*) and pro-opio melanocortin (*pomca*) was assessed by real time quantitative PCR (RT-qPCR), using the CFX384 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA), with the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) as described in Basto et al. [40]. Neuropeptide forms were selected based on previous reports of their involvement in the intake modulation in European seabass [36, 40]. Reactions were performed with 40–400 nM of each primer (forward and reverse; Table 3), 5 µL of Green Supermix and 2 µL of cDNA, achieving a total reaction volume of 10 µL. Thermal cycling conditions were as follows: 95 °C for 2 min, followed by 40 cycles of two steps—first, 95 °C for 5 s, then primer annealing temperature (60–62 °C; Table 3) for 28 s. After the final PCR cycle, post-amplification dissociation curves were systematically monitored (60–95, 0.5 °C in each cycle) to guarantee reaction specificity. PCR efficiency was evaluated in serial twofold dilutions of cDNA through from a sample pool of all experiments, using the CFX Maestro 2.3 software (Bio-Rad, Hercules, CA, USA). Only values between 90% and 115% were accepted. All samples were analysed in duplicate and blanks without cDNA were run as negative control.

Ribosomal protein 40S (*rps40*), elongation factor 1 alpha (*ef1α*) and beta-actin (*βact*) were considered as housekeeping genes; the most stable genes or combination of genes were assessed using the qbase^{+} Software, considering the lowest M index and CV. The Pfaffl method was applied for relative quantification of target gene transcripts [41].

Table 3 Oligonucleotide sequences used to assess relative mRNA quantity through RT-qPCR

Gene	Primer sequence	Annealing temperature (°C)	PCR efficiency (%)	Accession number
agrp2	F: GGGCAGAGGACACAAAGAAA R: TGTGACTTTCCTGTGGTGGA	H: 62 T: 62	H: 114 T: 108	HE660087
npy	F: ACGGAGGGATACCCGGTGAA R: GCTGAGTAGTACTTGGCCAGCTC	H: 62 T: 60	H: 110 T: 113	AJ005378
cartpt2	F: CCGAACCTGACCAGCGAGAA R: GCTCCCCGACATCACACGTT	H: 62 T: 60	H: 107 T: 107	MZ441181
pomca	F: CCGGTCAAAGTCTTCACCTC R: ACCTCCTGTGCCTTCTCCTC	H: 62	H: 112	AY691808
β-act	F: CAAAGCCAACAGGGAGAAGATGA R: ACCGGAGTCCATGACGATAC	H: 60 T: 60	H: 113 T: 93	AJ537421
rps40	F: TGATTGTGACAGACCCTCGTG R: CACAGAGCAATGGTGGGGAT	H: 62 T: 62	H: 109 T: 106	HE978789
ef1α	F: AACTTCAACGCCCAGGTCAT R: CTTCTTGCCAGAACGACGGT	H: 60 T: 62	H: 94 T:112	AJ866727.1

Statistical Analysis

Data were examined for normality and homogeneity of variances according to Kolmogorov–Smirnov and Levene's tests, respectively, and, if needed, adequately transformed. Two-way ANOVA was applied to analyze data, using the Statistica software. If significant effects of treatments were found, means were compared through the pairwise Tukey multiple comparison test. When data did not comply with the ANOVA assumptions, a Kruskal–Wallis test was performed for each factor and the pairwise multiple comparison of mean ranks was carried out to identify significant differences between groups.

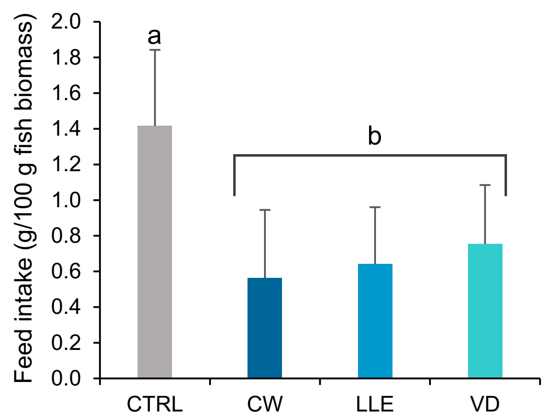


Fig. 1 Feed intake of fish fed the experimental diets after one meal. Values presented as mean + SD ($n = 6$). Different letters above the bars indicate significant differences among dietary treatments ($p < 0.05$)

Results

Extracts Characterization

LLE-A was the extract richest in aldehydes and organic acids, while VD-A displayed the highest amounts of alcohols and ketones (Table 1). No organic acids were observed in CW-A and VD-A extracts. Moreover, alkanes and sulphur compounds were only present in the CW-A and undetected in the processed aroma extracts. 1-Penten-3-ol was the most abundant compound in all fractions and, as expected, supplemented diets displayed similar levels of this compound and well above those found in the CTRL diet (0.9 vs. 2.1; Table 1).

Feed Intake

Figure 1 depicts the feed intake after the first single meal with the experimental diets. No significant differences could be found among supplemented diets and they all resulted in a significantly lower intake than that of the CTRL diet (0.5–0.8 vs. 1.4 g/100 g).

Plasma Metabolites

Plasma metabolites concentrations are reported on Table 4. No significant differences were observed for any of the analysed parameters, considering dietary treatments, sampling time and the interaction between these factors. However, the CW diet led to slightly higher plasma concentrations of NEFA, cholesterol and triglycerides, 2 h after the first feeding; these tendencies disappeared at the 6 h sampling point, where the CW diet actually led to slightly lower values of NEFA and triglycerides.

Table 4 Plasma metabolites of European seabass, 2 or 6 h after the first feeding with the experimental diets

	2 h				6 h				*p*-value		
	CTRL	CW	LLE	VD	CTRL	CW	LLE	VD	Diet	Sampling time	Interaction
Glucose	4.402 ± 0.582	6.281 ± 0.477	4.689 ± 0.395	5.014 ± 0.585	6.977 ± 0.616	4.116 ± 0.504	6.145 ± 0.765	5.246 ± 0.712	0.884	0.602	0.203
Lactate	3.969 ± 0.537	4.318 ± 0.810	3.582 ± 0.356	4.708 ± 0.457	4.085 ± 0.541	3.574 ± 0.819	4.642 ± 0.713	4.208 ± 0.578	0.827	0.968	0.456
NEFA	0.463 ± 0.186	1.474 ± 0.373	0.557 ± 0.183	0.467 ± 0.195	0.974 ± 0.296	0.411 ± 0.150	1.605 ± 0.375	0.881 ± 0.289	0.607	0.142	0.032*
Cholesterol	3.161 ± 0.541	4.995 ± 0.399	2.924 ± 0.282	3.427 ± 0.476	4.687 ± 0.592	3.250 ± 0.331	4.802 ± 0.725	5.151 ± 0.861	0.873	0.051	0.060
Triglycerides	8.246 ± 1.232	13.784 ± 1.641	7.430 ± 1.235	7.699 ± 1.522	8.708 ± 1.684	7.448 ± 1.654	12.355 ± 1.588	9.232 ± 1.595	0.429	0.893	0.052

Values presented as mean + SE ($n = 6$), in mmol L^{-1}

*Without significant differences after post-hoc test

Intake-Regulating Neuropeptides

No statistically significant differences were observed for the expression of neuropeptides involved in the regulation of feed intake, neither in the hypothalamus nor in the telencephalon (Fig. 2; Table 5). However, supplemented diets displayed a slight increase in the expression of orexigenic *agrp* on the telencephalon, 6 h after the feeding, compared to the 2 h sampling point. LLE also showed a similar tendency for the expression of *npy* in the hypothalamus. Regarding anorexigenic neuropeptides, the CTRL, CW and LLE diets displayed an increase in *cartpt* expression in the hypothalamus, 6 h after the feeding, a tendency which was not observed in the telencephalon. It was not possible to quantify *pomc* in the telencephalon, due to its low expression levels. In the hypothalamus, the expression of this neuropeptide was highest for the LLE diet.

Discussion

This work had the dual objectives of extracting aromas from sardine cooking wastewaters within the context of a circular economy and evaluating the influence of incorporating these aroma concentrate extracts into the diet on the short-term feed intake modulation of European seabass.

The developed aroma concentrate extracts were predominantly composed of alcohols and aldehydes, mostly derived from the breakdown of sardine oil during steaming [2]. The chemical aroma profile of the CW-A corresponds well to previous analysis of sardine cooking wastewaters supplemented with acorn extract [6]. Yet, some differences could be perceived as no quantifiable amounts of 1-octanol and hexanoic acid were observed in the present work, whilst pentanal was the third most abundant aldehyde in CW-A, contrarily to the previous study. The further processing of the cooking wastewaters extracts aimed at obtaining a fraction richer in aromas and without impurities. However, this caused losses in some compounds, including most aldehydes and all pentenols. In contrast, the vacuum distillation process led to an extract with higher concentration of long-chain alcohols (such as octanol and decanol) and ketones, while the LLE-A displayed higher amounts of organic acids. This suggests that aldehydes and short-chain alcohols (such as pentenols) are probably more prone to losses during processing. Most of these losses are thought to occur during the centrifugation and the liquid–liquid extraction or vacuum distillation phases. The reverse osmosis is precisely applied in order to have a more concentrated fraction prior to these latter procedures. This is confirmed by the higher concentrations of 1-penten-3-ol obtained by Pereira et al. [6], after reverse osmosis applied to the sardine cooking waters. Comparing the two processes, liquid–liquid extraction and

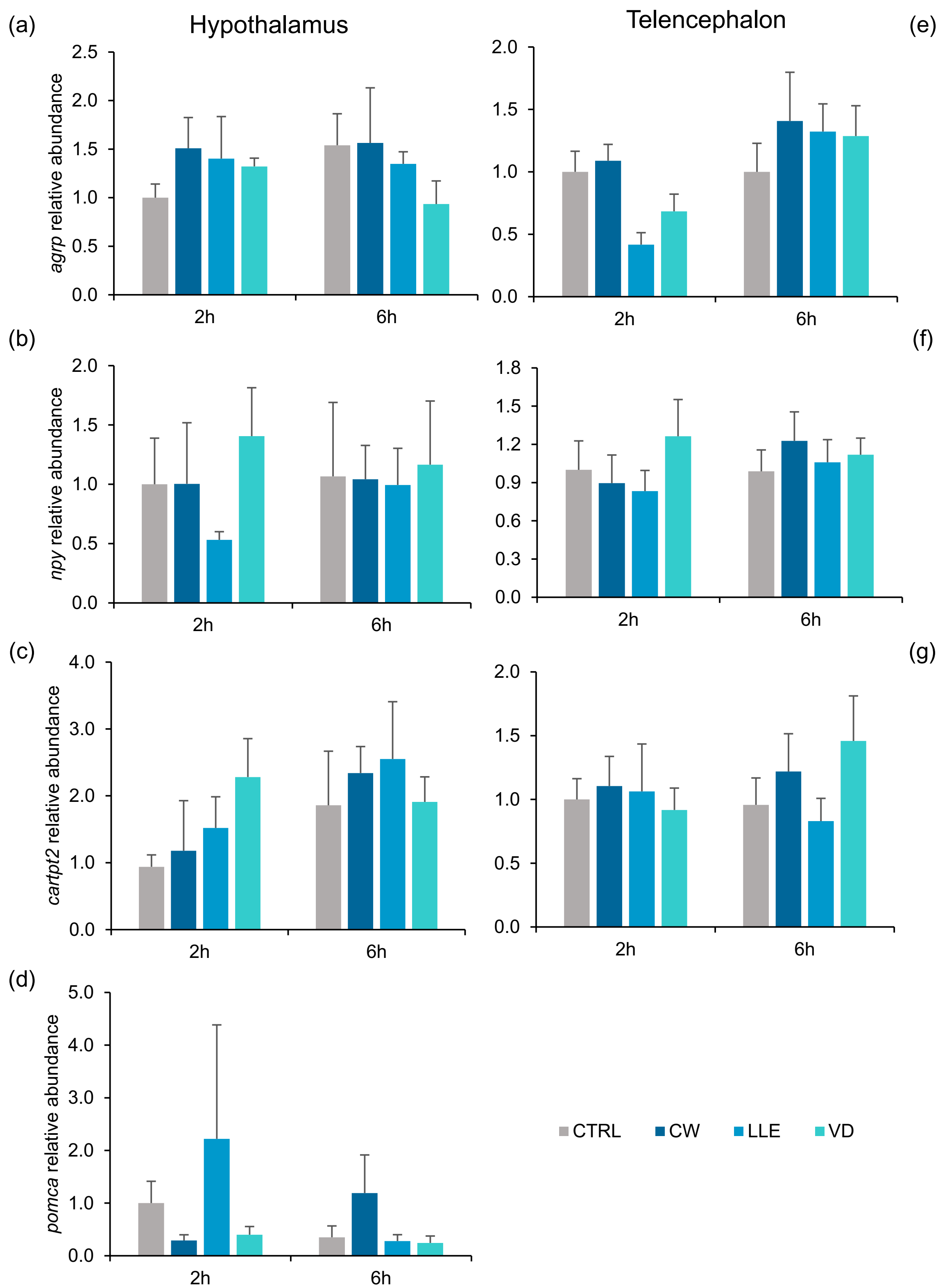


Fig. 2 Post-feeding mRNA abundance, in the hypothalamus (**a**–**d**) and telencephalon (**e**–**g**), of neuropeptides related to feed intake modulation of European seabass, after the first meal with the experimental diets. Results are presented as average + SEM ($n = 6$)

Table 5 *p*-Values of the two away ANOVA analysis of the neuropeptide expression in the hypothalamus and telencephalon of fish fed the experimental diets

	Neuropeptide	Diet	Sampling time	Diet × Sampling time
Hypothalamus	*agrp2*	0.602	0.857	0.604
	npy	0.811	0.550	0.273
	cartpt2	0.412	0.143	0.958
	pomca	0.560	0.336	0.489
Telencephalon	*agrp2*	0.439	0.008	0.062
	npy	0.791	0.482	0.921
	cartpt2	0.635	0.716	0.881

vacuum distillation, we observe that generally the VD-A displayed a higher concentration of several compounds, including the 1-penten-3-ol and 2,4-heptadienal, and thus seems more promising. This could be due to these compounds' lower hydrophobicity. However, it is important to note that the vacuum distillation process implies a cooling step, potentially increasing its price and energetic expenditure. Moreover, the aroma recovering processes are usually optimized considering the removal of off-flavours [42], generally defined based on literature regarding human sensory perception. Future studies in fish regarding the animals' response to individual aroma compounds, in order to better understand what is an off-flavour to the animals, would aid in the optimization of these processes towards application in aquafeeds. In any case, the developed processes allowed the successful removal of alkanes and sulphur-based compounds, generally classified as off-flavours and as having a "green beans" aroma [43, 44], from the CW-A, as they were not detected in the final extracts.

The European seabass is a carnivorous fish species, and thus the dietary inclusion of extracts rich in compounds such as hexanal, 2,4-heptadienal, 1-octen-3-ol, and 1-penten-3-ol, described as having a "fishy" or "meaty" odour [6, 45], could possibly be an innovative way to increase feed intake of plant-based diets. However, fish's response to odorants may differ from that of mammals, and thus not elicit this expected response [27, 46]. Senzui and Fukada [47] showed that supplementing a *Seriola quinqueradiata* diet with alanine, an amino acid with strong olfactory stimulant properties, promotes a "search" response in fish, without increasing feed intake. This suggests that olfactive properties of diet alone may not be enough to modulate the complex final response of feed intake. This could be one of the reasons that led to the worse response of fish to the aroma-supplemented diets, as reflected by the worse VFI displayed by these diets. In any case, these intake values are consistent with previous literature reports for this species fed traditional fishmeal diets [36]. The control diet led to VFI values which were significantly higher than all supplemented values, suggesting that the supplementation with these aromas was not effective at increasing feed intake. Studies using aroma enriched extracts as feed intake modulators are scarce, and thus understanding the reasons behind this poor efficacy is difficult. However, some literature reports have been performed, mostly based on electrophysiological studies. Aliphatic acids, particularly those with 3–5 carbons, have been reported as having stimulatory properties on Atlantic salmon (*S. salar*), common carp (*Cyprinus carpio*) and Japanese eel (*Anguilla japonica*) [28, 48], but long and medium chain fatty acids decrease feed intake [49, 50]. The LLE-A was the only extract in which fatty acids with lower carbon chains were found, but the hexanoic acid may already be deterrent, as observed by the lower VFI of this diet. Additionally, the CW-A had high levels of pentanal and 2,4-heptadienal, which may present a "rubber" and "rancid" odour [51], possibly being detrimental off-flavours in fish diets and hence resulting in decreased feed intake. Furthermore, most of the volatile compounds present in the tested extracts are a result of fish oil degradation during sardine cooking [2]. As such, and although they may have a "fish" odour to humans, they might be recognized by fish as being associated with rancidity rather than fresh fish, and thus act as deterrent. In this study, the response after a single meal was assessed. This approach avoids habituation responses, and also minimizes the effects of metabolic needs that are more influential in long-term trials, compared to the hedonic regulation of feed intake [40].

Analysis of neuropeptides in the brain regions most associated with feed intake regulation can provide information of the impact of dietary formulations on fish appetite. In this work, no statistically significant differences were observed among dietary treatments or sampling times, on both tissues assessed (telencephalon and hypothalamus). Likewise, in *Rachycentron canadum*, diets with different lysine to arginine rations that affected feed intake resulted in similar levels of *npy* expression [52]. In a short trial, variations in neuropeptide levels pre-feeding will be due to circadian response and would not provide additional information, as the fish have not been previously exposed to the experimental diets. However, in a longer trial, after daily exposed to the experimental diets, this approach may allow us to obtain information related to dietary-derived differences from a hedonic point of view. Additionally, it is worth noting that feed intake regulation depends on a complex integration of several neuropeptides and hormones, and thus the combination of non-significant changes in the individual expression of neuropeptides may result in significant alterations in the overall regulation of feed intake. In this sense, the LLE and VD diets displayed lower expression of the orexigenic *agrp* in the telencephalon, 2 h after the feeding, which may help explain the lower voluntary feed intake reported in fish fed such diets. Yet, this could not be observed 6 h after feeding, which suggests that the expression of this neuropeptide might have already returned to its basal levels. In fact, at 6 h post-feeding, the expression of neuropeptides, in both brain tissues, seems rather similar among diets. This is in agreement with a previous report on Atlantic salmon (*S. salar*), in which expression of all neuropeptides in the brain was similar in unfed fish (representing basal levels) and 6 h after the feeding [53]. The higher expression of the anorexigenic *pomca* in the hypothalamus of fish fed LLE, 2 h after feeding, also partially explains the lower feed intake of this diet, when compared to the control. A similar rationale can be applied to the higher expression of the anorexigenic of *cartpt* in the hypothalamus of fish fed LLE, 2 h after feeding, compared to the control.

Stress has a clear impact on appetite regulation [54]. However, it should be noted that glucose and lactate levels, common stress indicators in fish [55], were statistically similar among diets and within expected values for this species [56]. Thus, it is not expected for the decrease of feed intake to be a result of stress of changing into a new diet. Feed intake is also modulated by nutrient metabolism (homeostatic regulation) [25]. Nevertheless, since diets were isolipidic, isoproteic and isoenergetic, and provide all essential amino acids and fatty acids for European seabass, it is not expected that alterations on feed intake are due to a consequence of homeostatic regulation, at least in the short-term. All fish had been previously fed the same diets for the whole quarantine and habituation period, and they should be at a similar metabolic state, which is unlikely to change drastically immediately after a single first meal. Moreover, in the literature, reports of short response times to changes in feed exist, probably due to an hedonic preference [57]. The results found for the plasma metabolites partially corroborate this hypothesis, as no significant differences on major metabolites (glucose, lactate, NEFA, cholesterol and triglycerides) were observed for any dietary treatments, either 2 or 6 h after feeding. This indicates a similar nutrient metabolism of all diets. Moreover, 6 h after feeding, the CW diet displays slightly lower levels of all plasma metabolites but displays a slight overexpression of anorexigenic neuropeptides. This suggests that the hedonic regulation is prevailing over the homeostatic regulation, since despite a decrease in circulating nutrients, fish were not responding with a potential increase on appetite. However, further analysis of putative hedonic signalling mechanisms, including markers from the endocannabinoid and opioid systems, along with dopamine or serotonin, are suggested in future work to verify this hypothesis. Something similar happened with the LLE diet, but 2 h after feeding—lower levels of plasmatic metabolites accompanied with an increase in the anorexigenic *pomca* and a decrease in orexigenic *npy*. It is also worth noting that these situations are similar, despite occurring at different post-prandial moments. In spite of the lack of significant changes in plasma metabolites between the two selected sampling points, 2 and 6 h post-prandial, in the long term these tendencies may lead to a significant effect on nutrient metabolism and possibly affect homeostatic intake modulation. Moreover, some aroma compounds, for example, heptanal, 2,4-heptadienal, 2,6-nonadienal, (E,Z) and 2-nonanone, often display antimicrobial activity [58–63] and could hence lead to microbiota alterations with a direct impact on nutrient metabolism. Therefore, a modulation of feed intake through homeostatic processes might occur, and at a longer term, this should be taken into account when assessing overall growth performance.

Further analysis of other molecules also involved in feed intake modulation, at central and peripheral level, such as ghrelin, cholecystokinin and orexins should also be performed in order to obtain a clearer picture of the impact of these diets on feed intake modulation, along with markers from the endocannabinoid and opioid systems, dopamine or serotonin, which are believed to be involved in the regulation of feed intake in fish. Analysis of fish feed behaviour could also shed light on whether these extracts promote search behaviours and if it is worth pursuing further optimization of these extracts.

Conclusions

Overall, we were able to extract several volatile compounds from sardine cooking wastewaters, highlighting the potential for waste valorisation. However, the supplementation of plant-based aquafeeds with such compounds, at the tested concentration, did not result in a positive short-term feed intake response for European seabass, neither considering the application of a direct cooking wastewaters, nor after further processing. To understand the full potential of these extracts to improve the feeding preferences of European seabass, further optimization of the extracts' production process, and/or of their inclusion levels in diets for European seabass should be considered. Additionally, it is worth noting that while the extracts did not yield the desired effects in European seabass, they may hold promise as attractants for other fish species or even for different animal models, and that hypothesis merits further evaluation.

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supervision, project administration, funding acquisition, writing—review and editing; CV: Conceptualization, methodology, validation, supervision, writing—original draft, writing—review and editing. All authors approved the final manuscript.

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Data Availability Data will be made available on request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical Approval The animal experimental trial was approved by the CIIMAR ethical committee for Managing Animal Welfare (ORBEA) and conducted by accredited scientists in laboratory animal science by the Portuguese Veterinary Authority (1005/92, DGAV-Portugal), following FELASA category C recommendations. The trial was performed following the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals for scientific purposes.

References

1. INE (Instituto Nacional de Estatística): Estatísticas da Pesca 2022. Instituto Nacional de Estatística, Lisbon, Portugal. https://www.dgrm.mm.gov.pt/esta (2022)
2. Ferraro, V., Carvalho, A.P., Piccirillo, C., Santos, M.M., Castro, P.M.L., Pintado, M.E.: Extraction of high added value biological compounds from sardine, sardine-type fish and mackerel canning residues—a review. Mater. Sci. Eng. C (2013). https://doi.org/10.1016/j.msec.2013.04.003
3. Garcia-Sanda, E., Omil, F., Lema, J.M.: Clean production in fish canning industries: recovery and reuse of selected wastes. Clean Technol. Environ. Policy (2003). https://doi.org/10.1007/s10098-003-0200-4
4. Artiga, P., García-Toriello, G., Méndez, R., Garrido, J.M.: Use of a hybrid membrane bioreactor for the treatment of saline wastewater from a fish canning factory. Desalination (2008). https://doi.org/10.1016/j.desal.2007.01.112
5. Carrera, P., Casero-Díaz, T., Castro-Barros, C.M., Méndez, R., Val del Río, A., Mosquera-Corral, A.: Features of aerobic granular sludge formation treating fluctuating industrial saline wastewater at pilot scale. J. Environ. Manag. (2021). https://doi.org/10.1016/j.jenvman.2021.113135
6. Pereira, M.J., Grosjean, O., Pintado, M., Brazinha, C., Crespo, J.: Clean technologies for production of valuable fractions from sardine cooking wastewaters: an integrated process of flocculation and reverse osmosis. Clean Technol. (2022). https://doi.org/10.3390/cleantechnol4020016
7. Naylor, R.L., et al.: A 20-year retrospective review of global aquaculture. Nature (2021). https://doi.org/10.1038/s41586-021-03308-6
8. Francis, G., Makkar, H.P.S., Becker, K.: Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture (2001). https://doi.org/10.1016/S0044-8486(01)00526-9
9. Gatlin, D.M., III., et al.: Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res. (2007). https://doi.org/10.1111/j.1365-2109.2007.01704.x
10. Geurden, I., et al.: The positive impact of the early-feeding of a plant-based diet on its future acceptance and utilisation in rainbow trout. PLoS ONE (2013). https://doi.org/10.1371/journal.pone.0083162
11. Liang, X., et al.: Growth and feed intake regulation responses to anorexia, adaptation and fasting in Japanese seabass, *Lateolabrax japonicas* when fishmeal is totally replaced by plant protein. Aquaculture (2019). https://doi.org/10.1016/j.aquaculture.2018.09.010
12. Sabioni, R.E., Lorenz, E.K., Cyrino, J.E.P., Volkoff, H.: Feed intake and gene expression of appetite-regulating hormones in *Salminus brasiliensis* fed diets containing soy protein concentrate. Comp. Biochem. Phys. A. (2022). https://doi.org/10.1016/j.cbpa.2022.111208
13. Torstensen, B.E., et al.: Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. Aquaculture (2008). https://doi.org/10.1016/j.aquaculture.2008.08.025
14. Prem, R., Tewari, V.K.: Development of human-powered fish feeding machine for freshwater aquaculture farms of developing countries. Aquac. Eng. (2020). https://doi.org/10.1016/j.aquaeng.2019.102028
15. Dauda, A.B., Ajadi, A., Tola-Fabunmi, A.S., Akinwole, A.O.: Waste production in aquaculture: sources, components and managements in different culture systems. Aquac. Fish. (2019). https://doi.org/10.1016/j.aaf.2018.10.002
16. Kokou, F., Fountoulaki, E.: Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. Aquaculture (2018). https://doi.org/10.1016/j.aquaculture.2018.06.003
17. Soengas, J.L., Cerdá-Reverter, J.M., Delgado, M.J.: Central regulation of food intake in fish: an evolutionary perspective. J. Mol. Endocrinol. (2018). https://doi.org/10.1530/jme-17-0320
18. Delgado, M.J., Cerdá-Reverter, J.M., Soengas, J.L.: Hypothalamic integration of metabolic, endocrine, and circadian signals in fish: involvement in the control of food intake. Front. Neurosci. (2017). https://doi.org/10.3389/fnins.2017.00354
19. Lin, X., Volkoff, H., Narnaware, Y., Bernier, N.J., Peyon, P., Peter, R.E.: Brain regulation of feeding behavior and food intake in fish. Comp. Biochem. Phys. A. (2000). https://doi.org/10.1016/S1095-6433(00)00230-0
20. Hu, J., et al.: Comparative transcriptome analysis of olfactory epithelium in large yellow croaker: evidence for olfactory adaptation to feed phagostimulant in fish. Aquaculture (2020). https://doi.org/10.1016/j.aquaculture.2020.734920

21. Comesaña, S., Velasco, C., Conde-Sieira, M., Míguez, J.M., Soengas, J.L., Morais, S.: Feeding stimulation ability and central effects of intraperitoneal treatment of L-leucine, L-valine, and L-proline on amino acid sensing systems in rainbow trout: implication in food intake control. Front. Physiol. (2018). https://doi.org/10.3389/fphys.2018.01209
22. Soengas, J.L.: Integration of nutrient sensing in fish hypothalamus. Front. Neurosci. (2021). https://doi.org/10.3389/fnins.2021.653928
23. Díaz-Rúa, A., Chivite, M., Comesaña, S., Conde-Sieira, M., Soengas, J.L.: The opioid system in rainbow trout telencephalon is probably involved in the hedonic regulation of food intake. Front. Physiol. (2022). https://doi.org/10.3389/fphys.2022.800218
24. Díaz-Rúa, A., Chivite, M., Comesaña, S., Velasco, C., Valente, L.M.P., Soengas, J.L., Conde-Sieira, M.: The endocannabinoid system is affected by a high-fat-diet in rainbow trout. Horm. Behav. (2020). https://doi.org/10.1016/j.yhbeh.2020.104825
25. Conde-Sieira, M., Soengas, J.L.: Nutrient sensing systems in fish: impact on food intake regulation and energy homeostasis. Front. Neurosci. (2017). https://doi.org/10.3389/fnins.2016.00603
26. Dias, J., Gomes, E.F., Kaushik, S.: Improvement of feed intake through supplementation with an attractant mix in European bass fed plant-protein rich diets. Aquat. Living Resour. (1997). https://doi.org/10.1051/alr:1997043
27. Morais, S.: The physiology of taste in fish: potential implications for feeding stimulation and gut chemical sensing. Rev. Fish. Sci. Aquac. (2017). https://doi.org/10.1080/23308249.2016.1249279
28. Kasumyan, A.O., Døving, K.B.: Taste preferences in fishes. Fish Fish. (2003). https://doi.org/10.1046/j.1467-2979.2003.00121.x
29. Chotikachinda, R., Tantikitti, C., Benjakul, S., Rustad, T., Kumarnsit, E.: Production of protein hydrolysates from skipjack tuna (*Katsuwonus pelamis*) viscera as feeding attractants for Asian seabass (*Lates calcarifer*). Aquac. Nutr. (2013). https://doi.org/10.1111/anu.12024
30. Chen, M., Chen, X., Nsor-Atindana, J., Masamba, K.G., Ma, J., Zhong, F.: Optimization of key aroma compounds for dog food attractant. Anim. Feed Sci. Technol. (2017). https://doi.org/10.1016/j.anifeedsci.2016.12.005
31. Takács, S., Musso, A.E., Gries, R., Rozenberg, E., Borden, J.H., Brodie, B., Gries, G.: New food baits for trapping house mice, black rats and brown rats. Appl. Anim. Behav. Sci. (2018). https://doi.org/10.1016/j.applanim.2017.11.011
32. Utne-Palm, A.C., Bogevik, A.S., Humborstad, O.-B., Aspevik, T., Pennington, M., Løkkeborg, S.: Feeding response of Atlantic cod (*Gadus morhua*) to attractants made from by-products from the fishing industry. Fish. Res. (2020). https://doi.org/10.1016/j.fishres.2020.105535
33. Ganeko, N., et al.: Analysis of volatile flavor compounds of sardine (*Sardinops melanostica*) by solid phase microextraction. J. Food Sci. (2008). https://doi.org/10.1111/j.1750-3841.2007.00608.x
34. Resende, D., et al.: Innovative swine blood hydrolysates as promising ingredients for European seabass diets: impact on growth performance and resistance to *Tenacibaculum maritimum* infection. Aquaculture (2022). https://doi.org/10.1016/j.aquaculture.2022.738657
35. Kır, M., Sunar, M.C., Gök, M.G.: Acute ammonia toxicity and the interactive effects of ammonia and salinity on the standard metabolism of European sea bass (*Dicentrarchus labrax*). Aquaculture (2019). https://doi.org/10.1016/j.aquaculture.2019.734273
36. Basto, A., Valente, L.M.P., Soengas, J.L., Conde-Sieira, M.: Partial and total fishmeal replacement by defatted *Tenebrio molitor* larvae meal do not alter short- and mid-term regulation of food intake in European sea bass (*Dicentrarchus labrax*). Aquaculture (2022). https://doi.org/10.1016/j.aquaculture.2022.738604
37. Comesaña, S., Velasco, C., Ceinos, R.M., López-Patiño, M.A., Míguez, J.M., Morais, S., Soengas, J.L.: Evidence for the presence in rainbow trout brain of amino acid-sensing systems involved in the control of food intake. Am. J. Physiol. Regul. Integr. Comp. Physiol. (2018). https://doi.org/10.1152/ajpregu.00283.2017
38. Velasco, C., Conde-Sieira, M., Comesaña, S., Chivite, M., Míguez, J.M., Soengas, J.L.: Role of the G protein-coupled receptors GPR84 and GPR119 in the central regulation of food intake in rainbow trout. J. Exp. Biol. (2021). https://doi.org/10.1242/jeb.242360
39. Ferreira, M., et al.: Diets supplemented with *Saccharina latissima* influence the expression of genes related to lipid metabolism and oxidative stress modulating rainbow trout (*Oncorhynchus mykiss*) fillet composition. Food Chem. Toxicol. (2020). https://doi.org/10.1016/j.fct.2020.111332
40. Basto, A., Valente, L.M.P., Conde-Sieira, M., Soengas, J.L.: Central regulation of food intake is not affected by inclusion of defatted *Tenebrio molitor* larvae meal in diets for European sea bass (*Dicentrarchus labrax*). Aquaculture (2021). https://doi.org/10.1016/j.aquaculture.2021.737088
41. Pfaffl, M.W.: A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. (2001). https://doi.org/10.1093/nar/29.9.e45
42. Pereira, M.J., Pintado, M., Brazinha, C., Crespo, J.: Recovery of valuable aromas from sardine cooking wastewaters by pervaporation with fractionated condensation: matrix effect and model validation. Membranes (2022). https://doi.org/10.3390/membranes12100988
43. Xu, M., Jin, Z., Lan, Y., Rao, J., Chen, B.: HS-SPME-GC-MS/olfactometry combined with chemometrics to assess the impact of germination on flavor attributes of chickpea, lentil, and yellow pea flours. Food Chem. (2019). https://doi.org/10.1016/j.foodchem.2018.12.048
44. Karolkowski, A., Guichard, E., Briand, L., Salles, C.: Volatile compounds in pulses: a review. Foods. (2021). https://doi.org/10.3390/foods10123140
45. Giri, A., Osako, K., Ohshima, T.: Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. Food Chem. (2010). https://doi.org/10.1016/j.foodchem.2009.10.036
46. Hamdani, E.H., Døving, K.B.: The functional organization of the fish olfactory system. Prog. Neurobiol. (2007). https://doi.org/10.1016/j.pneurobio.2007.02.007
47. Senzui, A., Fukada, H.: Olfaction and gustatory senses promote feeding through different pathways in yellowtail, *Seriola quinqueradiata*. Aquaculture (2023). https://doi.org/10.1016/j.aquaculture.2022.738814
48. Marui, T., Caprio, J.: Teleost gustation. In: Hara, T.J. (ed.) Fish Chemoreception, pp. 171–198. Chapman & Hall, Winnipeg, Canada (1992)
49. Feng, H., Peng, D., Liang, X.-F., Li, J., Luo, H., Tang, S., Chai, F.: Intracerebroventricular injection with octanoic acid activates hypothalamic fatty acid sensing systems and regulates appetite in Chinese perch *Siniperca chuatsi*. Fish. Sci. (2022). https://doi.org/10.1007/s12562-021-01570-1
50. Velasco, C., Conde-Sieira, M., Comesaña, S., Chivite, M., Díaz-Rúa, A., Míguez, J.M., Soengas, J.L.: The long-chain fatty acid receptors FFA1 and FFA4 are involved in food intake regulation in fish brain. J. Exp. Biol. (2020). https://doi.org/10.1242/jeb.227330
51. Venkateshwarlu, G., Let, M.B., Meyer, A.S., Jacobsen, C.: Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. J. Agric. Food Chem. (2004). https://doi.org/10.1021/jf034833v
52. Nguyen, M.V., Jordal, A.-E.O., Espe, M., Buttle, L., Lai, H.V., Rønnestad, I.: Feed intake and brain neuropeptide Y (NPY) and

cholecystokinin (CCK) gene expression in juvenile cobia fed plant-based protein diets with different lysine to arginine ratios. Comp. Biochem. Phys. A. (2013). https://doi.org/10.1016/j.cbpa.2013.04.004

53. Valen, R., Jordal, A.E.O., Murashita, K., Rønnestad, I.: Postprandial effects on appetite-related neuropeptide expression in the brain of Atlantic salmon, *Salmo salar*. Gen. Comp. Endocrinol. (2011). https://doi.org/10.1016/j.ygcen.2011.02.027
54. Santos, G.A., Schrama, J.W., Mamauag, R.E.P., Rombout, J.H.W.M., Verreth, J.A.J.: Chronic stress impairs performance, energy metabolism and welfare indicators in European seabass (*Dicentrarchus labrax*): the combined effects of fish crowding and water quality deterioration. Aquaculture (2010). https://doi.org/10.1016/j.aquaculture.2009.11.018
55. Fanouraki, E., Mylonas, C.C., Papandroulakis, N., Pavlidis, M.: Species specificity in the magnitude and duration of the acute stress response in Mediterranean marine fish in culture. Gen. Comp. Endocrinol. (2011). https://doi.org/10.1016/j.ygcen.2011.06.004
56. Silva-Brito, F., Timóteo, F., Esteves, Â., Peixoto, M.J., Ozorio, R., Magnoni, L.: Impact of the replacement of dietary fish oil by animal fats and environmental salinity on the metabolic response of European seabass (*Dicentrarchus labrax*). Comp. Biochem. Physiol. B (2019). https://doi.org/10.1016/j.cbpb.2019.04.004
57. Carlberg, H., Cheng, K., Lundh, T., Brännäs, E.: Using self-selection to evaluate the acceptance of a new diet formulation by farmed fish. Appl. Anim. Behav. Sci. (2015). https://doi.org/10.1016/j.applanim.2015.08.016
58. Wood, W.F., Szewczak, J.M.: Volatile antimicrobial compounds in the pelage of the Mexican free-tailed bat, *Tadarida brasiliensis mexicana*. Biochem. Syst. Ecol. (2007). https://doi.org/10.1016/j.bse.2007.04.002
59. Abarca, R.L., et al.: Application of β-cyclodextrin/2-nonanone inclusion complex as active agent to design of antimicrobial packaging films for control of *Botrytis cinerea*. Food Bioprocess Tech. (2017). https://doi.org/10.1007/s11947-017-1926-z
60. Li, S.-F., Zhang, S.-B., Lv, Y.-Y., Zhai, H.-C., Hu, Y.-S., Cai, J.-P.: Heptanal inhibits the growth of *Aspergillus flavus* through disturbance of plasma membrane integrity, mitochondrial function and antioxidant enzyme activity. LWT (2022). https://doi.org/10.1016/j.lwt.2021.112655
61. Ma, W., Johnson, E.T.: Natural flavour (E,E)-2,4-heptadienal as a potential fumigant for control of *Aspergillus flavus* in stored peanut seeds: finding new antifungal agents based on preservative sorbic acid. Food Control (2021). https://doi.org/10.1016/j.foodcont.2021.107938
62. Tanaka, K., Taniguchi, S., Tamaoki, D., Yoshitomi, K., Akimitsu, K., Gomi, K.: Multiple roles of plant volatiles in jasmonate-induced defense response in rice. Plant Signal. Behav. (2014). https://doi.org/10.4161/psb.29247
63. Cho, M.J., Buescher, R.W., Johnson, M., Janes, M.: Inactivation of pathogenic bacteria by cucumber volatiles (E,Z)-2,6-nonadienal and (E)-2-nonenal. J. Food Prot. (2004). https://doi.org/10.4315/0362-028X-67.5.1014

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4.2. Incorporating sardine cooking water aromas into plant-based diets for European seabass: effects on appetite regulation, growth and sensory properties of fish flesh

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Incorporating sardine cooking water aromas into plant-based diets for European seabass: effects on appetite regulation, growth and sensory properties of fish flesh

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Abstract

Sardine cooking wastewaters are by-products of the canning industry with great potential for valorisation. We have hypothesized that they can be a source of aromas to enhance appetite when added to plant-based diets. The poor palatability of such diets often described in carnivorous species poses a recurring problem in fish farming, with harsh consequences on fish growth performance and flesh quality. Aromas from sardine cooking wastewaters were collected without processing (CW-A), processed through vacuum distillation (VD-A), or processed through liquid/liquid extraction with soybean oil (LLE-A) into plant-based diets. Each aroma was added to a plant-protein based diet for European seabass, at a concentration of 2 µg of 1-penten-3-ol/g diet, resulting in 3 experimental diets (CW, VD and LLE). A non-supplemented diet was used as a control. Each diet was assigned to triplicate fish groups (initial weight 95.7 g), that were hand-fed twice daily until apparent satiation in a recirculating saltwater system at 21 °C. After 18 weeks, fish growth performance and nutrient utilisation were evaluated. The expression in the brain of neuropeptides involved in feed intake regulation was also analysed. Moreover, flesh colour and texture were assessed instrumentally and by sensory analysis using a consumer panel. Fish fed LLE displayed a significantly higher feed intake than those fed CW which was correlated with an increased neuropeptide Y expression in the hypothalamus. However, LLE slightly hinders lipid metabolism, leading to lower available glucose and resulting in statistically similar final weights among diets. Despite variations in fillet hardness, the sensory panel revealed similar overall liking across all treatments. The findings indicate that aromas from sardine cooking wastewaters can modulate feed intake, but further refinement in processing or incorporation levels is required to potentiate their efficacy.

Keywords

Circular economy; European seabass; aromas; feed intake; appetite stimulants; palatability.

1. Introduction

Plant-based ingredients have been considered suitable options for replacing marine-based ingredients, such as fishmeal (FM) and fish oil (FO) in aquafeeds, since these have become scarce, expensive and environmentally unsustainable (Naylor et al., 2021). However, using plant ingredients as the main source of protein for farmed marine carnivorous species presents several challenges. Feed intake can be reduced when high levels of plant ingredients are included in aquafeeds, ultimately affecting fish growth, health or even sensorial properties. Torstensen et al. (2008) showed a reduced intake in Atlantic salmon (*Salmo salar*) smolts fed a diet with 80% of plant proteins and 70% vegetable oils, compared to a marine-based aquafeed, for the first three months of feeding these experimental diets. Likewise, in European seabass (*Dicentrarchus labrax*), Dias et al. (1997) observed a reduced feed intake when fed diets devoid of FM, relying on soy protein concentrates as main protein sources. Finally, Torrecillas et al. (2017) observed a reduction of feed intake of European seabass, after 90 days of feeding diets with 20% FM and 6% FO, compared with a diet with 58% FM and 15% FO. In fish farms, the feed can account for up to 40-60% of the production costs (Prem and Tewari, 2020) and is also responsible for a large portion of the waste production in this sector, either due to uneaten feed or dietary digestibility issues (Kokou and Fountoulaki, 2018; Dauda et al., 2019). Therefore, both the sustainability of aquaculture and the quality of its end products will depend, among other factors, on improvements in diet formulation, a greater understanding of the fish behaviour and physiology, and dedicated research focused on improving feed intake and efficiency.

In fish, feed intake modulation depends on signals relating to energetic needs (homeostatic) and to the pleasant sensation of eating (hedonic) (Soengas et al., 2018). Integration of both hedonic and homeostatic signals occurs in the central nervous system, mainly in the hypothalamus and telencephalon regions (Lin et al., 2000; Delgado et al., 2017; Soengas, 2021; Díaz-Rúa et al., 2022). This integration results in the regulation of certain neuropeptides which directly influence feed intake. These neuropeptides can be anorexigenic (reduce feed intake), such as the cocaine and amphetamine-related transcript (CART) and the pro-opiomelanocortin (POMC) or orexigenic (increase feed intake), such as the agouti-related peptide (AgRP) and neuropeptide Y (NPY) (Comesaña et al., 2018a; Soengas, 2021). To increase feed intake of plant-based diets, which are generally less palatable than marine-based ones, modulation of the hedonic regulation of feed intake is needed, and one option for that is the use of feed attractants. The hedonic regulation of feed intake relies on sensorial and rewarding signals and it may even prevail over homeostatic regulation of intake, which can lead to feed consumption to levels that surpass those needed to cover all energy requirements (Comesaña et al., 2018b; Díaz-Rúa et al., 2022).

Several ingredients have been proposed as feed attractants for aquafeeds including amino acids (Kasumyan and Døving, 2003), peptides/hydrolysates (Chotikachinda et al., 2013), betaine (Mackie et al., 1980; Lim et al., 2016), nucleotides and nucleosides (Hossain et al., 2020). However, very little is yet known regarding other compounds such as alcohols, aldehydes and organic acids, which are known to promote food intake in mammals (Chen et al., 2017; Takács et al., 2018). Hence, the search for new feed attractants is still very relevant. In addition to its effectiveness, feed attractants must be economically and environmentally sustainable. The utilization of agri-food by-products as a source of such compounds holds the potential to contribute to circular economy policies (Utne-Palm et al., 2020).

In the canning industry, the production of one tonne of canned fish can generate as much as 9 m^3 of liquid waste. Notably, wastewaters produced during the cooking process are very rich in volatile compounds. However, these wastewaters must be processed prior to disposal to minimize environmental impact, which can be both complex and costly (Ferraro et al., 2013). Volatiles released during the cooking of fish in canning industries are currently not being recovered but once appropriately extracted, they have the potential to become valuable compounds, particularly when incorporated in aquafeeds. Beyond their potential as feed attractants, supplementing farmed fish diets with such volatiles can also help shape the fish's aroma profile, ultimately enhancing its appeal to consumers. However, to the best of our knowledge, no previous study assessed the impact of dietary inclusion of volatiles on the sensory profile of fish fillets. It is worth nothing that dietary changes have been reported to influence fish taste, flavour or texture (Schlüter et al., 1999; Luo et al., 2017; Ma et al., 2020). In addition, the replacement of marine ingredients by plant-based alternatives in diets may have implications for flesh quality (Fountoulaki et al., 2009). Therefore, the impact of diets with high levels of plant-based ingredients should always be considered.

Thus, the objective of this study was to explore the potential of incorporating aromas from sardine cooking wastewaters into plant-based diets for European seabass, a carnivorous species reliant on high levels of FM in its diet. The study aimed to investigate the effectiveness of these aromas as feed intake modulators and to assess their ability to enhance the organoleptic characteristics of fish fillets.

2. Materials and methods

2.1. Ethical issues

The experimental trial, previously approved by the CIIMAR ethical committee for Managing Animal Welfare (ORBEA-CIIMAR_18_2017), was performed by accredited scientists in laboratory animal science by the Portuguese Veterinary Authority (1005/92, DGAV-Portugal, following FELASA category C recommendations) and conducted according to the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals for scientific purposes.

2.2. Aromas production

Sardine cooking wastewaters (A Poveira S.A., Laúndos, Portugal) were supplemented with a 1% antioxidant extract (acorn extract) to minimize aroma deterioration. This cooking wastewater aroma, designated as CW-A, underwent additional processing as described by Resende et al. (2024). Initially, it was subjected to centrifugation to obtain an aqueous fraction, which was further treated though reverse osmosis. The resulting fraction underwent two distinct processes: liquid-liquid extraction with soybean oil, producing the LLE-A aroma, and vacuum distillation, resulting in the VD-A aroma (Resende et al., 2024).

2.3. Diet preparation

Four isonitrogeneous (52% protein in dry matter, DM), isolipidic (18% DM) and isoenergetic (22 kJ/g) diets were developed: a practical plant-based diet, with 12.5% FM and 4% FO, was defined as the control (CTRL); the three test diets (CW, LLE, VD) were obtained by adding each aroma to the CTRL (table 1). The incorporation level of each aroma (CW-A, LLE-A, VD-A) into its corresponding diet (CW, LLE, VD) was set as to include the same amount of 1-penten-3-ol in all diets (2 µg/g diet). This compound was the most abundant in all extracted aromas and is responsible for the flavour of fresh marine products (Ganeko et al., 2008; Pereira et al., 2022a). All tested diets were formulated in conformity with European seabass nutrient requirements (National Research Council, 2011). All diets were formulated and extruded by SPAROS Lda. (Olhão, Portugal), using a pilot-scale twin-screw extruder (CLEXTRAL BC45, France), with a screw diameter of 55.5 mm, keeping temperature at 105–110 °C. The resulting extrudes, without oil, were oven-dried (OP 750-UF, LTE Scientifics, United Kingdom), for 3 h, at 60 °C. To avoid degradation of the extracts upon extrusion, each extract was added to the oil fraction and incorporated into extruded diets under vacuum conditions, in a Pegasus vacuum coater (PG-10VCLAB, DINNISEN, Netherlands). Experimental diets were then stored at 4 °C until use. Ingredients and proximate composition of diets are described on table 1, reflecting the ingredient amounts considering the post-extrusion addition of the oils.

Table 1 – Formulation and chemical composition of diets used in the growth trial.

	Diets			
	CTRL	CW	LLE	VD
Ingredients (g/kg)				
Fishmeal [a]	125.0	125.0	125.0	125.0
Soy protein concentrate [b]	250.0	250.0	250.0	250.0
Wheat gluten [c]	150.0	150.0	150.0	150.0
Corn gluten meal [d]	100.0	100.0	100.0	100.0
Soybean meal [e]	110.0	110.0	110.0	110.0
Wheat meal [f]	91.0	91.0	91.0	91.0
Vitamin and mineral premix [g]	5.0	5.0	5.0	5.0
DCP [h]	15.0	15.0	15.0	15.0
Fish oil [i]	40.0	40.0	40.0	40.0
Soybean oil [j]	114.0	114.0	76.5	114.0
CW-A (mL) [k]		12.00		
LLE-A [l]			37.5	
VD-A (mL) [m]				100.00
Chemical composition (%DM)				
Dry matter	95.24	94.53	95.08	94.53
Ash	6.52	6.51	6.57	6.51
Crude protein	52.19	52.28	52.15	52.28
Crude fat	18.12	17.87	17.58	17.87
Energy (kJ/g)	22.65	22.84	22.68	22.84
Phosphorus	0.85	0.86	0.87	0.86
1-Penten-3-ol (µg/g)	0.98	2.10	2.16	2.10

[a] Fishmeal NORVIK LT, Sopropêche, France (72% crude protein, 7% crude fat); [b] Soy protein concentrate Soycomil®-P, ADM, Animal Nutrition ™, Netherlands (65% protein, 0.7% lipids); [c] Wheat gluten composition: DM: 901 g kg^{-1}; protein: 838 g kg^{-1}; lipids: 16 g kg^{-1}; [d] Corn gluten feed from COPAM, Portugal (61% crude protein, 6% crude fat); [e] Dehulled solvent extracted soybean meal, CARGILL, Spain (48% crude protein, 2.2% crude fat); [f] Wheat meal from Casa Lanchinha Lda., Portugal (10.2% protein, 1.2% lipids); [g] INVIVO 1%, Premix for marine fish, PREMIX Lda, Portugal; [h] Di-calcium phosphate; [i] Sardine oil, Sopropêche, France; [j] Henry Lamotte Oils, GmbH, Germany; [k] Extract from sardine cooking wastewaters; [l] Extract from sardine cooking wastewaters processed through liquid/liquid extraction; [m] Extract from sardine cooking wastewaters processed through vacuum distillation.

2.4. Growth trial

Juvenile European seabass were transported from a commercial fish farm (Atlantik Fish Lda, Portugal) to the Fish Culture Experimental Unit of CIIMAR (Matosinhos, Portugal). Fish were quarantined for 2 weeks in a 2000 L tank included in a recirculating saltwater system (RAS) and fed a commercial diet (Aquasoja, Sorgal S.A.; 50% crude protein, 20% crude fat, as DM basis) once daily. Nitrogenous compounds (NH_4^+ ≤0.05 mg L^{-1}; NO_2^- ≤ 0.5 mg L^{-1}; NO_3^- ≤ 5 mg L^{-1}) salinity (35‰), temperature (21 ± 1 °C), dissolved oxygen (>90% saturation) and pH ($7.5 \leq pH \leq 8.5$) were frequently examined and kept at recommended levels for this species (Kır et al., 2019). A cycle of 12 h light/12 h dark was set as photoperiod.

After quarantine, fish were individually weighed (95.7 ± 13.5 g) and measured (total length, 20.4 ± 1.0 cm) and 12 homogeneous groups of 15 fish were randomly distributed (initial density of 7 kg m^{-3}) into 200 L fiberglass tanks within a RAS (10 L min^{-1} flow rate). Environmental conditions were kept as described for the quarantine period. Each diet was randomly assigned to triplicate groups of fish that were fed to apparent satiation, twice a day by hand, for 18 weeks. Feed intake was calculated by weighing the given feed and subtracting any leftover feed collected from the tanks. At the beginning of the trial, 5 fish from the initial stock were collected after a 24 h fasting period, sacrificed by anaesthetic overdose (0.5 mL L^{-1} of 2-phenoxyethanol, Sigma-Aldrich, MO, USA), and stored at −80 °C, for initial whole-body composition analysis. An intermediate sampling was conducted at 7 weeks, in which fish were bulk weighed to monitor weight gain and register feed consumption.

At the end of the 18-week period, two fish per tank were collected after a 6-h fasting, sacrificed by anaesthetic overdose (2-phenoxyethanol), and individually weighed (g) and measured (total length, cm). Blood was collected with heparinized syringes and centrifuged at 5000 g for 10 min at 4 °C. The plasma was stored at -80 °C until metabolites analysis. Hypothalamus and telencephalon were also collected and flash-frozen in dry ice prior to storage at -80 °C until molecular biology analysis. The instrumental quantification of colour and texture was carried out in fresh dorsal muscle samples from those fish. After a 48-h fasting period the remaining 13 fish per tank were collected and individually weighed (g) and measured (total length, cm). Three fish per tank were sacrificed by anaesthetic overdose (2-phenoxyethanol) and stored at −80 °C, for whole-body composition analysis, and 10 fish per tank were sacrificed by ice bath and transported in styrofoam boxes with ice to SenseTest, Lda facilities for sensory analysis.

2.5. Chemical analysis

To analyse whole-body composition, three fish from each tank were pooled and ground and dry matter was determined (105 °C, 24 h). Then, pooled samples were freeze-dried. Diets were ground prior to chemical analysis.

Ground and dried samples were analysed according to AOAC methods, as described in Resende et al. (2022). Briefly, dry matter (105 °C, 24 h), ash (combustion in a muffle furnace, Nabertherm L9/11/B170, Bremen, Germany; 550 °C, 6 h), crude protein (N ×

6.25; Leco nitrogen analyser FP-528, Leco Corporation, St. Joseph, USA), crude fat (petroleum ether extraction; Soxtec^{TM} 2055, FOSS, Höganäs, Sweden), gross energy (adiabatic bomb calorimeter; Werke C2000, IKA, Staufen, Germany) and phosphorus (acid digestion of ashes followed by quantification of phosphates using ammonium molybdate and absorbance reading at 820 nm) were determined in duplicate. Additionally, in aromatic extracts and diets, SPME/GC-MS (solid phase microextraction followed by gas chromatography-mass spectrometry) was performed for identification of the aroma compounds present in the extracts and quantification of 1-penten-3-ol, using the method described in Pereira et al. (2022a). A Shimadzu gas chromatograph (GCMS-QP2010) equipped with a WAX column (30 m x 0.25 mm i.d. x 0.25 µm) was used during the study. Ultrapure helium at 1 mL min $^{-1}$ was used as the carrier gas. The oven temperature program was: 60 °C (held for 4 min), followed by an increase of 2 °C min^{-1} to 180 °C. The injector temperature was set at 200 °C, limited by the temperature of desorption indicated by the SPME fibre manufacturer. Detector temperature was set at 220 °C, ionization source at 200 °C and the ionization mode was electron impact with electron energy of 70 eV. A volume of 6 mL was then extracted by CAR/PDMS fibre during 15 min to 60 °C with and without stirring. The time of analyte desorption from the SPME fibre was fixed at 10 min. The injection was performed in the spitless mode for 2 min. After this time, the split ratio was set at 1:20 until the final of the chromatographic run. 2-nonanol was used as an internal standard for analysis of the sample. To obtain a good extraction of the tested compounds, 2 g of NaCl were added to all the samples analysed.

2.6. Metabolite's analysis

All liver samples were homogenised with 0.6 M perchloric acid and neutralized with 1 M potassium bicarbonate, as described in Basto et al. (2022). After centrifugation (10000 g, 4.5 min), the supernatant was collected and used for metabolites quantification. Plasma samples were deproteinized with 0.6 M perchloric acid, followed by neutralization with potassium bicarbonate and centrifugation at 13500 g for 4.5 min. Metabolites quantification was performed in triplicate on a microplate spectrophotometer (BioTek Synergy HT, Vermont, USA), using commercial kits (glucose, lactate, triglycerides, total lipids and cholesterol: Spinreact, Barcelona, Spain; non-esterified fatty acids, NEFA: Wako Chemicals, Neuss, Germany), adapted to microplate, as described in Velasco et al. (2021).

2.7. Quantification of mRNA abundance of intake-regulating neuropeptides

Whole hypothalamus or telencephalon were used for total RNA extraction with Trizol reagent (Invitrogen, Carlsbad, CA, USA), in accordance with the manufacturer´s recommendations (with some modifications), followed by purification using a NZY Total RNA Isolation Kit (NZYTech, Lisbon, Portugal), as reported in Ferreira et al. (2020). RNA purity and quantity was assessed with a DeNovix DS-11FX spectrophotometer (Wilmington, DE, USA), considering the absorbance ratio 260:280 nm. If the A260/A280 ratio was in the range of 1.80–2.20, samples were deemed suitable for analysis. Afterwards, 1.5 µg of RNA was reverse transcribed to cDNA, using a NZY First-Strand cDNA Synthesis Kit (NZYTech, Lisbon, Portugal), according to the manufacturer's instructions.

Neuropeptide Y (*npy*), agouti-related peptide (*agrp2*), cocaine and amphetamine-related transcript (*cartpt2*) and pro-opio melanocortin (*pomca*) expression was evaluated by real time quantitative PCR (RT-qPCR), using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA), with the CFX384 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The forms of the neuropeptides assessed in this study were chosen based on reports by Basto et al. (2021); Basto et al. (2022), considering previous reports of their roles as either orexigens or anorexigens. All reactions were carried out with primer (forward and reverse) concentrations of 40 – 400 nM, plus 5 µL of Green Supermix and 2 µL of cDNA, totalling a reaction volume of 10 µL. The applied thermal cycling conditions were: 95 °C for 2 min, followed by 40 cycles of two steps – first, 95 °C for 5 s; second, primer annealing temperature (60–62 °C) for 28 s (table 2). After the final PCR cycle, post-amplification dissociation curves were systematically monitored (60–95, 0.5 °C in each cycle), to warrant reaction specificity. PCR efficiency was analysed in serial 2-fold dilutions of cDNA using a sample pool of all experiments, with the CFX Maestro 2.3 software (Bio-Rad, Hercules, CA, USA). Values between 90 and 115% were considered suitable (the R^2 for all genes assessed was higher than 0.92). Samples were assessed in duplicate. The negative control was set as blanks without cDNA. Table 2 describes the primer sequence, annealing temperatures, PCR efficiencies and accession numbers for all analysed genes.

For housekeeping genes, ribosomal protein 40S (*rps40*), elongation factor 1 alpha (*ef1α*) and beta-actin (*βact*) were considered. With the qbase+ software, the most stable genes or combination of genes were determined, according to the lowest M index and CV. The Pfaffl method was then employed for relative quantification of target gene transcripts (Pfaffl, 2001).

Table 2 – Oligonucleotide sequences, efficiency and annealing temperature used for relative mRNA quantification through RT-qPCR.

Gene	Primer Sequence	Annealing temperature (°C)	PCR Efficiency (%)	Accession number
agrp2	F: GGGCAGAGGACACAAAGAAA	H: 62	H: 109	HE660087
	R: TGTGACTTTCCTGTGGTGGA	T: 62	T: 97	
npy	F: ACGGAGGGATACCCGGTGAA	H: 60	H: 110	AJ005378
	R: GCTGAGTAGTACTTGGCCAGCTC	T: 60	T: 111	
cartpt2	F: CCGAACCTGACCAGCGAGAA	H: 62	H: 105	MZ441181
	R: GCTCCCCGACATCACACGTT	T: 60	T: 106	
pomca	F: CCGGTCAAAGTCTTCACCTC	H: 62	H: 103	AY691808
	R: ACCTCCTGTGCCTTCTCCTC			
β-act	F: CAAAGCCAACAGGGAGAAGATGA	H: 62	H:102	AJ537421
	R: ACCGGAGTCCATGACGATAC	T: 60	T: 104	
rps40	F: TGATTGTGACAGACCCTCGTG	H: 62	H:103	HE978789
	R: CACAGAGCAATGGTGGGGAT	T: 62	T: 91	
ef1α	F: AACTTCAACGCCCAGGTCAT	H: 60	H: 107	AJ866727.1
	R: CTTCTTGCCAGAACGACGGT	T: 62	T: 97	

2.8. Instrumental texture and colour

Instrumental colour and texture were evaluated as described in Pereira et al. (2022b). Briefly, skin and muscle colour were analysed with a CR-400 chroma meter (Konica Minolta Inc., Osaka, Japan), with respect to CIE standard illuminant D65. A white plate reference standard (Minolta Co, Ltd., Osaka, Japan) was used for calibration of the apparatus. Colour parameters were determined by applying the colorimeter onto either skin or raw fillets from 6 fish per dietary treatment, in three points above the lateral line of each fillet. After flashing, *CIE-L* a*b** values were registered. Fillet texture was assessed using a TA.XT.plus Texture Analyser (Stable Micro Systems Inc., Godalming, United Kingdom) with a 5 kg load cell and a 2.0 mm diameter probe. Hardness (N), adhesiveness (J), springiness, cohesiveness, chewiness (J) and resilience measurements were acquired by double compression (constant speed and penetration depth of 1 mm s^{-1} 320 and 4.0 mm, respectively) on the thickest portion of each raw fillet. Penetration depth was set according to the maximum distance that did not produce fibre breakage.

2.9. Consumer profiling and liking

The "rate-all-that-apply" (RATA) methodology (Ares et al., 2014; Baião et al., 2022) was applied to evaluate consumer profiling of the seabass samples. The list of terms in the RATA ballot were generated by the research team, combining previous qualitative consumer studies with published studies from evaluation of samples performed by a trained panel (Montero et al., 2005; Mendes and Gonçalves, 2008; Makol et al., 2013). Table 3 describes the final list of sensory attributes used in the RATA ballot. Then, overall liking using the classic 9-point hedonic scale (Peryam and Pilgrim, 1957) was assessed with a panel, made of 75 regular consumers of fish (minimum of one fish meal per week), recruited from the sensory evaluation company Sense Test's consumer database (Vila Nova de Gaia, Portugal). They were mostly residents in the Oporto metropolitan area (north of Portugal). The company ensures data protection and confidentiality following the EU General Data Protection Regulation (EU 2016/679), along with an established internal code of conduct, guaranteeing informed consent. Sensory evaluation was performed in individual tasting booths at a special room equipped according to ISO 8589:2007 - Sensory analysis - General guidance for the design of test rooms.

For sensory evaluation, gut and scales of European seabass were removed. Heads, tails and fins were cut off and fish were divided into three slices (anterior, middle and posterior). Each slice was wrapped in microperforated aluminium foil and steamed in a preheated industrial forced convector oven Rational Combi-Master CM61, Rational AG, Germany), for 10 min at 100 °C. Each consumer evaluated slices from the same part of the fish (anterior, middle or posterior) across all samples.

The participants were asked to carry out a RATA evaluation with a list of 24 sensory attributes divided into four dimensions (table 3): appearance (5), odour (5), texture/mouth-feel (7) and taste (7). Panellists were asked to mark the terms they thought applicable for describing samples and then to rate the intensity of each selected attribute, with a 5-point scale (from 1 = "slightly applicable" to 5 = "very applicable").

Table 3 – List of sensory attributes used in the "rate-all-that-apply" ballot (original in Portuguese). Attributes were classified as either absent or present and if present, the respective intensity was rated using a 5-point structured scale (from 1 = "slightly applicable" to 5 = "very applicable").

Sensory dimension	**Sensory attributes**
Appearance (*Aparência*)	Moist (*húmido*), juicy (*suculento*), white flesh (*carne branca*), dark flesh in the middle (*carne escura no interior*), glossy flesh (*carne brilhante*)
Odour (*Odor*)	Fish smell (*a peixe*), sea smell (*a mar/maresia*), musty (*a mofo*), soft/weak (*suave/pouco intenso*), fresh (*fresco*)
Texture/Mouth-feel (*Textura*)	Slimy (*mole*), dry (*seca*), fibrous (*fibrosa*), juicy (*suculenta*), tender (*macia*), firm (*firme*), easy to flake (*fácil de lascar*)
Taste (*Sabor*)	Earthy (*a terra*), soft (*suave*), seabass characteristic (*típico a robalo*), intense (*intenso*), balanced (*equilibrado*), persistent (*persistente*), musty (*a mofo*)

2.10. Calculations

Condition index (K) = 100 × final weight, g / (final length, cm)^3

Daily growth index (DGI) = 100 × [(final body weight, g)$^{1/3}$ – (initial body weight, g)$^{1/3}$]/ trial duration, d

Average body weight (ABW, kg) = (initial body weight, kg + final body weight, kg) / 2

Voluntary feed intake (VFI, g kg^{-1}d^{-1}) = dry nutrient intake, g / (ABW × trial duration, d)

Feed conversion ratio (FCR) = dry feed intake, g / weight gain, g

Hepatosomatic index (HSI) = liver weight, g / body weight, g × 100

Viscerosomatic index (VSI) = viscera weight, g / body weight, g × 100

Protein efficiency ratio (PER) = (final body weight, g – initial body weight, g) / (feed intake, g × protein amount in diets, %)

Nutrient gain (g kg^{-1} d^{-1}) = (final carcass nutrient content, g – initial carcass nutrient content, g) / (ABW × trial duration, d) × 100

Nutrient retention (%) = 100 × [(final body weight, g × final nutrient whole-body composition, %WW) – (initial body weight, g × initial nutrient whole-body composition, %WW)] / (feed intake, g × nutrient amount in diets, %)

2.11. Statistical analysis

All data were tested for normality and homogeneity of variances by Kolmogorov-Smirnov and Levene's tests, respectively, and adequately transformed if required. One-way ANOVA was applied to analyze data, using the SPSS (IBM SPSS Statistics 26, IL, USA) software. Whenever significant effects of treatments were detected, means were compared through the pairwise Tukey multiple comparison test. If data did not meet the assumptions of ANOVA, a Kruskal-Wallis test was carried out for each factor and the pairwise multiple comparison of mean ranks was performed to detect significant differences between groups. For the sensory analysis, a data set was created with the RATA applicability (0 = “not applicable”; 1 = “slightly applicable” to 5 = “very applicable”), as described in Baião et al. (2022)., RATA scores were calculated for each sample and term through the sum of the scores provided by the consumers who selected that term as applicable for that sample. A three-way ANOVA, considering dietary treatments, part of fish and consumer (set as random), was applied to identify which attributes were discriminating among samples ($p < 0.05$), followed by a pairwise multiple comparison test (Tukey HSD).

3. Results

3.1. Aromas and diets

The LLE-A displayed the highest amount of organic acids; VD-A had the highest concentrations of alcohols and ketones; finally, CW-A was richest in aldehydes (Figure 1). Organic acids were not found in the CW-A and VD-A. Among the compounds present, 1-penten-3-ol was found to be the most abundant in all aromas. As expected, the supplemented diets showed identical levels of this compound, which were well above those observed on the CTRL diet (0.9 vs 2.1; Table 1).

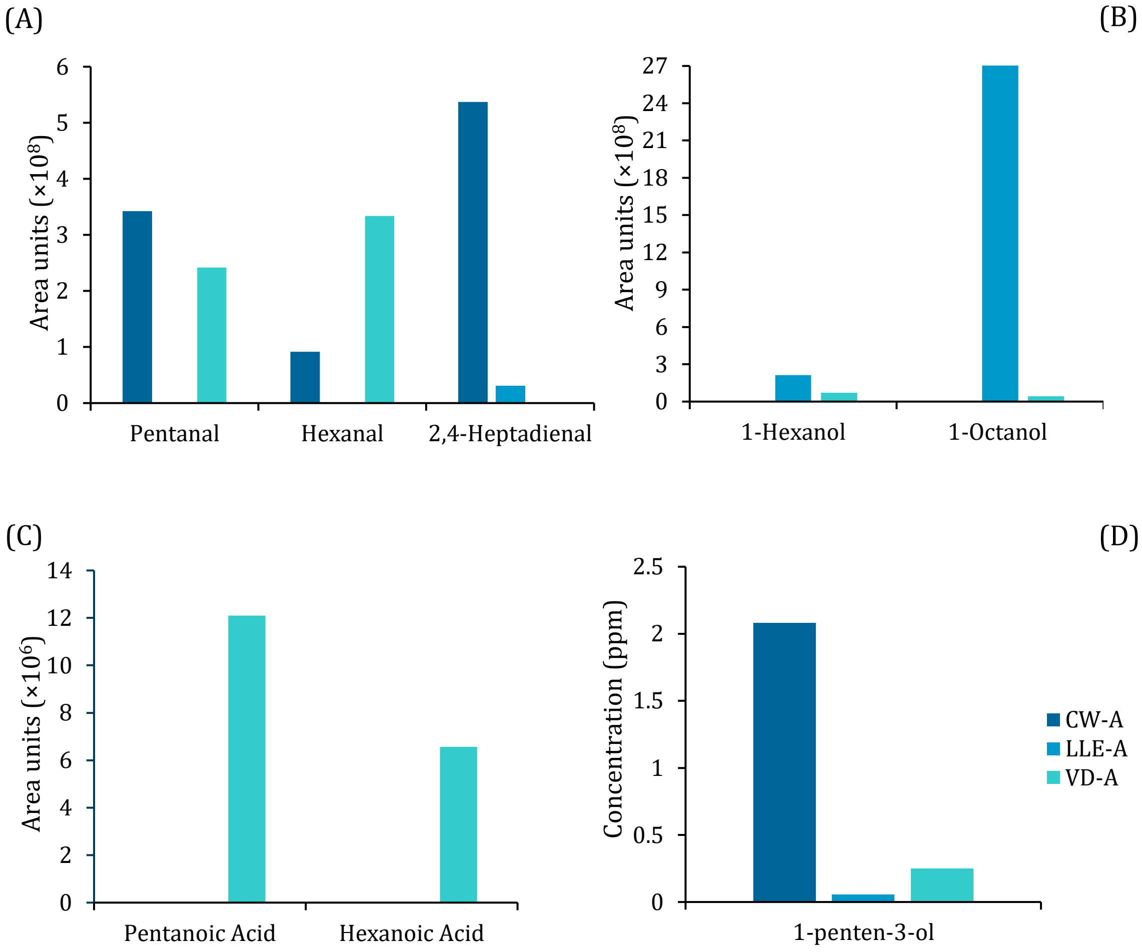

Figure 1 – Chemical profile of the different extracts (CW-A, LLE-A, VD-A): (A) main identified aldehydes; (B) main identified alcohols (excluding the marker compound); (C) main identified organic acids; (D) concentration of the compound set as marker, 1-penten-3-ol.

3.2. Growth performance

The fish doubled their weight during the growth trial (Table 4). However, the experimental diets did not result in any statistically significant differences ($p>0.05$) in the final weight, length, condition index, or daily growth index. The LLE-fed fish had the highest VFI and FCR. It significantly differed from those fed the CW diet ($p=0.026$). However, there were no significant differences between the LLE diet and the CTRL and VD diets for these parameters. The CW-fed fish displayed the highest HSI, but they differed only from the LLE group ($p=0.020$). The PER followed a similar trend ($p=0.028$). No differences were observed for the VSI among the fish fed the different dietary treatments ($p>0.05$).

Feeding fish the experimental diets did not lead to any significant differences ($p>0.05$) regarding final whole-body composition or nutrient gain and retention (Table 4).

Table 4 – Growth performance indicators and final whole-body composition of European seabass fed the experimental diets for 18 weeks.

	Diet				*p*-value
	CTRL	**CW**	**LLE**	**VD**	
Initial weight (g)	95.53 ± 13.25	95.75 ± 13.01	95.78 ± 14.21	95.63 ± 13.95	0.994
Final weight (g)	209.46 ± 39.09	206.56 ± 35.95	205.27 ± 37.39	210.28 ± 35.59	0.910
Initial length (cm)	20.4 ± 0.9	20.4 ± 0.9	20.4 ± 1.1	20.4 ± 0.9	>0.999
Final length (cm)	25.8 ± 1.3	25.9 ± 1.3	25.8 ± 1.4	26.0 ± 1.3	0.793
K	1.21 ± 0.09	1.17 ± 0.08	1.19 ± 0.07	1.18 ± 0.07	0.309
DGI	1.05 ± 0.02	1.03 ± 0.01	1.02 ± 0.04	1.06 ± 0.01	0.222
VFI (g/kg/day)	7.68 ± 0.21 ^{ab}	7.46 ± 0.03 ^{b}	7.98 ± 0.03 ^{a}	7.93 ± 0.16 ^{ab}	0.029
FCR	1.34 ± 0.01 ^{ab}	1.32 ± 0.01 ^{b}	1.43 ± 0.07 ^{a}	1.37 ± 0.02 ^{ab}	0.026
HSI (%)	1.57 ± 0.26 ^{ab}	2.06 ± 0.38 ^{a}	1.51 ± 0.32 ^{b}	1.55 ± 0.28 ^{ab}	0.020
VSI (%)	7.71 ± 0.89	8.16 ± 1.12	7.24 ± 1.26	7.57 ± 1.40	0.608
PER	1.43 ± 0.01 ^{ab}	1.45 ± 0.01 ^{a}	1.35 ± 0.07 ^{b}	1.39 ± 0.01 ^{ab}	0.028
Final whole-body composition (%WW)					
Moisture	62.36 ± 0.81	64.00 ± 0.61	62.43 ± 0.76	62.36 ± 1.48	0.182
Ash	3.17 ± 0.23	3.30 ± 0.16	3.05 ± 0.31	2.94 ± 0.24	0.353
Protein	18.22 ± 0.41	18.32 ± 0.67	19.09 ± 0.30	18.72 ± 0.57	0.215
Fat	17.04 ± 1.00	15.05 ± 0.81	16.69 ± 0.61	17.27 ± 1.68	0.134
Energy (kJ/g)	9.77 ± 0.25	9.43 ± 0.09	9.75 ± 0.30	10.02 ± 0.36	0.144
Phosphorus	0.50 ± 0.03	0.52 ± 0.04	0.49 ± 0.06	0.46 ± 0.05	0.630
Gain (g or kJ/kg ABW/day)					
Dry matter	2.21 ± 0.12	2.00 ± 0.06	2.15 ± 0.12	2.22 ± 0.16	0.176
Protein	1.04 ± 0.03	1.03 ± 0.06	1.10 ± 0.05	1.10 ± 0.07	0.288
Fat	1.05 ± 0.12	0.83 ± 0.08	0.99 ± 0.08	1.08 ± 0.18	0.121
Energy	56.69 ± 3.66	52.16 ± 1.25	55.09 ± 4.60	59.69 ± 4.32	0.171
Phosphorus	0.020 ± 0.003	0.022 ± 0.004	0.019 ± 0.006	0.017 ± 0.005	0.684
Retention / consumption (%)					
Dry matter	28.76 ± 0.86	26.77 ± 0.71	26.91 ± 1.67	28.01 ± 2.15	0.361
Protein	25.98 ± 1.19	26.57 ± 1.77	26.57 ± 1.27	26.51 ± 1.59	0.950
Fat	75.48 ± 7.11	62.17 ± 5.99	70.64 ± 5.63	76.37 ± 13.06	0.233
Energy	32.56 ± 1.31	30.74 ± 0.60	30.49 ± 3.02	32.95 ± 2.19	0.379
Phosphorus	31.39 ± 4.84	34.27 ± 6.29	27.02 ± 7.65	25.42 ± 7.78	0.411

Values presented as mean ± SD (n=3 except for final weight, length and K, in which n=45). Different superscript letters indicate significant differences between dietary treatments. Abbreviations: ABW – average body weight; DGI – daily growth index; FCR – feed conversion ratio; HSI – hepatosomatic index; K – condition index; PER - protein efficiency ratio; VFI – voluntary feed intake; VSI – viscerosomatic index.

3.3. Metabolites in plasma and liver

Regarding metabolites concentration in plasma, only glucose resulted in significant differences (p=0.033) among diets (Table 5). The CTRL diet led to higher plasma glucose levels compared to the LLE diet. Lactate, NEFA, cholesterol and triglycerides remained unaffected by dietary treatments (p>0.05). In the liver, glucose level was highest in the LLE-fed fish, which differed significantly (p=0.014) from the CW group (Table 5). The same tendency was observed for triglycerides (p=0.025). No differences among fish fed the different diets were observed in terms of liver glycogen, NEFA and total lipids (p>0.05).

Table 5 – Plasma and liver metabolite concentrations, in fish fed the experimental diets for 130 days, expressed in mmol L^{-1} and in µmol g $tissue^{-1}$, respectively.

	Diets				
	CTRL	**CW**	**LLE**	**VD**	***p*-value**
Plasma					
Glucose	6.418 ± 0.317 ^{a}	5.340 ± 0.472 ^{ab}	4.659 ± 0.364 ^{b}	5.407 ± 0.257 ^{ab}	0.033
Lactate	7.185 ± 0.259	5.822 ± 0.672	5.683 ± 0.867	6.295 ± 0.590	0.358
NEFA	0.982 ± 0.219	0.593 ± 0.211	0.367 ± 0.107	0.544 ± 0.151	0.218
Cholesterol	5.099 ± 0.532	4.943 ± 0.739	3.254 ± 0.569	5.195 ± 0.503	0.098
Triglycerides	14.518 ± 1.491	11.902 ± 2.916	11.282 ± 2.685	10.688 ± 2.158	0.684
Liver					
Glucose	22.04 ± 3.52 ^{ab}	13.70 ± 1.79 ^{b}	31.62 ± 4.18 ^{a}	20.09 ± 3.98 ^{ab}	0.014
Glycogen	99.32 ± 7.56	86.74 ± 3.94	92.23 ± 6.68	98.57 ± 5.84	0.447
NEFA	0.731 ± 0.310	0.176 ± 0.042	0.662 ± 0.108	0.853 ± 0.202	0.106
Triglycerides	3.07 ± 0.90 ^{ab}	2.08 ± 0.45 ^{b}	5.59 ± 1.08 ^{a}	3.15 ± 0.51 ^{ab}	0.025
Total lipids[1]	20.61 ± 4.02	18.11 ± 1.41	19.69 ± 3.81	23.50 ± 2.61	0.649

Values presented as mean ± SE (n=6). Different superscript letters indicate significant differences between dietary treatments. [1]Values presented in mg g^{-1}. Abbreviations: NEFA – non-esterified fatty acids.

3.4. Neuropeptide mRNA abundance on brain tissues

There were no significant differences (p>0.05) in neuropeptide expression observed in either the hypothalamus or telencephalon (Figure 2). However, in the hypothalamus, the LLE diet led to a higher *npy* expression compared to the CTRL and CW diets. The supplemented diets also appeared to slightly increase *agrp2* expression in the hypothalamus, although not significantly (p>0.05). Neuropeptide expression in the telencephalon showed more uniformity among diets. It was not possible to quantify *pomca* in the telencephalon due to its very low abundance. To assess the relationship between neuropeptide expression in both tissues and VFI, a Pearson correlation analysis was performed. The only significant correlation found was between *npy* expression in the hypothalamus and VFI, showing a positive correlation with a Pearson correlation coefficient of 0.762, and a p-value of 0.004.

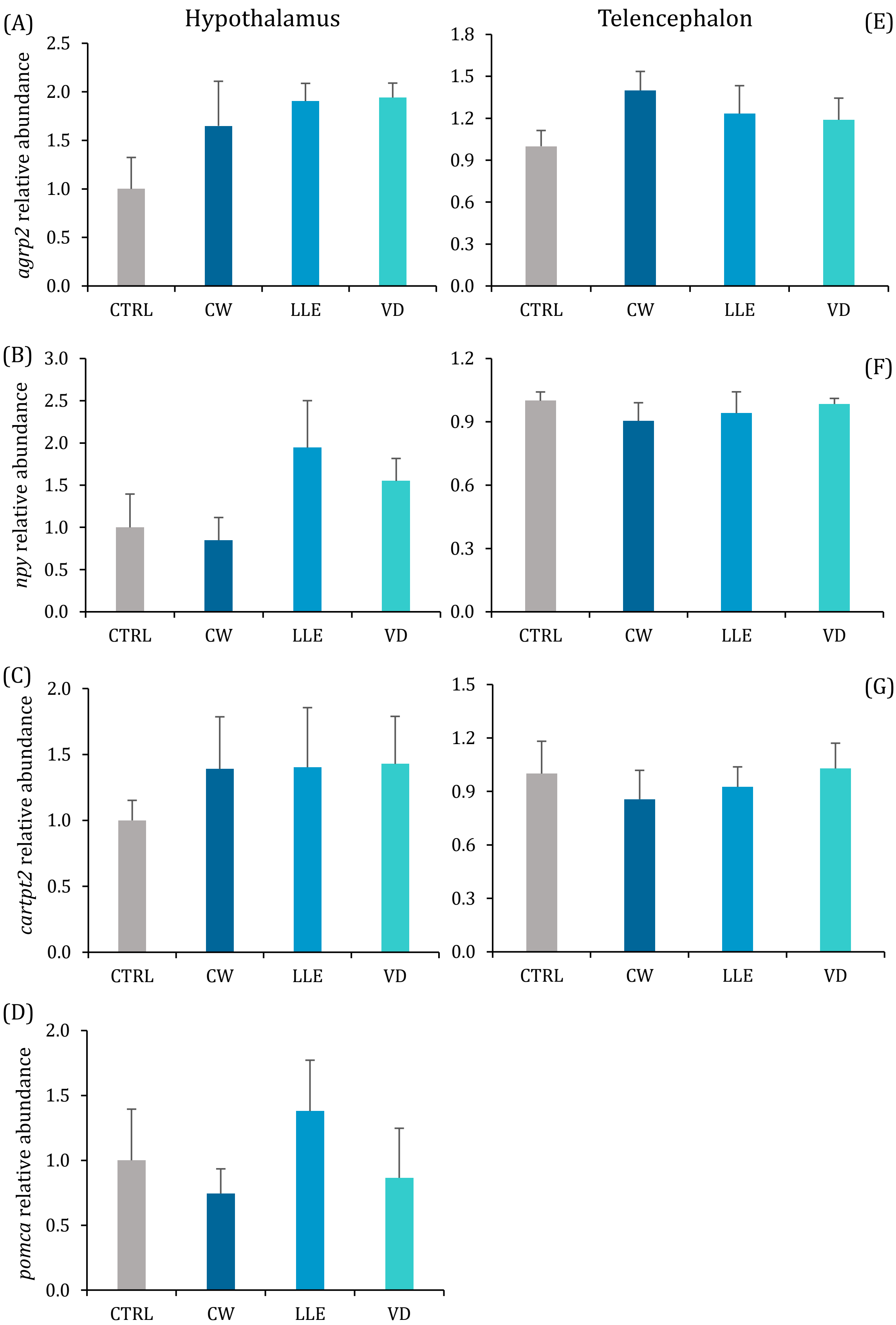

Figure 2 – Relative mRNA abundance of neuropeptides related to the feed intake regulation in European seabass fed the experimental diets (CTRL, CW, LLE or VD) for 18 weeks, in the hypothalamus (A-D) or telencephalon (E-G). Values are presented as average + SE (n=6).

3.5. Instrumentally assessed texture and colour properties

Table 6 depicts the instrumentally assessed colour properties of fish fillets and skin. Skin *L** values ranged from 38 to 41, *a** from -2.6 to -3.0 and *b** from 5.8 to 7.1. Regarding muscle colour, *L** values varied from 38 to 41, *a** from -1.8 to -2.2 and *b** from 3.0 to 5.1. There were no significant differences observed as a result of the dietary treatments (p>0.05).

Regarding fillet texture (Table 6), only hardness was significantly affected (p=0.006) by diets, with LLE leading to a lower hardness than CTRL; no other differences were observed among fillets from different dietary treatments (p>0.05).

Table 6 – Instrumental colour and texture analysis of skin and fillets of fish fed the experimental diets. Values presented as mean ± SD (n=6).

	Diet				***p*-value**
	CTRL	**CW**	**LLE**	**VD**	
Skin Colour					
*L**	38.93 ± 5.78	38.03 ± 2.45	41.16 ± 6.52	39.55 ± 6.44	0.797
*a**	-2.72 ± 0.73	-2.63 ± 0.44	-2.87 ± 0.28	-3.01 ± 0.75	0.685
*b**	5.89 ± 0.87	6.35 ± 0.91	7.06 ± 0.78	6.77 ± 1.30	0.216
Muscle colour					
*L**	40.75 ± 3.15	39.58 ± 0.98	38.44 ± 1.96	40.71 ± 1.33	0.190
*a**	-1.83 ± 0.26	-1.81 ± 0.45	-2.25 ± 0.45	-1.97 ± 0.40	0.282
*b**	5.08 ± 2.61	4.41 ± 1.05	3.08 ± 0.92	4.30 ± 1.61	0.130
Muscle texture					
Hardness (N)	0.914 ± 0.053 ^{a}	0.844 ± 0.085 ^{ab}	0.721 ± 0.078 ^{b}	0.745 ± 0.032 ^{ab}	0.006
Adhesiveness (J)	0.005 ± 0.003	-0.006 ± 0.009	-0.003 ± 0.015	0.007 ± 0.025	0.598
Springiness	1.042 ± 0.058	1.029 ± 0.075	1.091 ± 0.055	1.005 ± 0.055	0.813
Cohesiveness	0.541 ± 0.117	0.519 ± 0.046	0.514 ± 0.073	0.507 ± 0.062	0.888
Chewiness	0.520 ± 0.119	0.454 ± 0.070	0.406 ± 0.103	0.428 ± 0.086	0.229
Resilience	0.361 ± 0.046	0.365 ± 0.095	0.369 ± 0.091	0.359 ± 0.097	0.997

Different superscript letters indicate significant differences between dietary treatments.

3.6. Consumer sensory analysis

Samples across all dietary treatments displayed a similar sensory profile (Figure 3), with no statistically significant differences detected in any of the attributes (p>0.05). Regarding appearance, the most selected attributes were *juicy, moist* and *white flesh.* As for odour, it was mostly perceived as being *soft* and *fresh*. The texture was generally described as *soft* and *easy to chip*, while the taste was perceived as *soft, balanced* and *characteristic of seabass*. Attributes like *musty* odour and taste, as well as *earthy* taste, were barely perceived. The liking of seabass samples was high and remained unaffected (p>0.05) by the dietary treatments (Figure 4), with all treatments receiving over 93% positive responses and a median overall liking score of 8 out of 9.

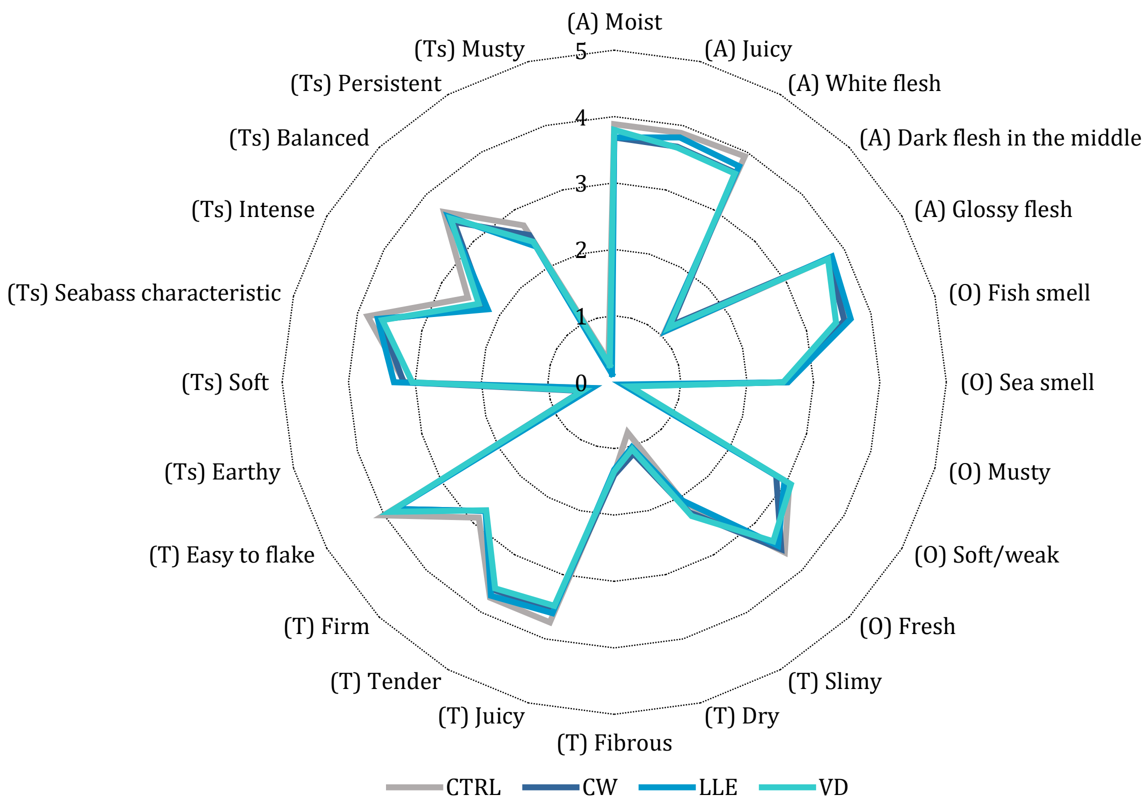

Figure 3 – Radar plot with average ratings of the sensory attributes (0 if unselected, otherwise rated on a scale from 1 to 5, with 1 = "slightly applicable" to 5 = "very applicable") of the fish fillets. Sensory attributes are ordered by sensory dimension: A - appearance, O – odour, T – texture and Ts – taste.

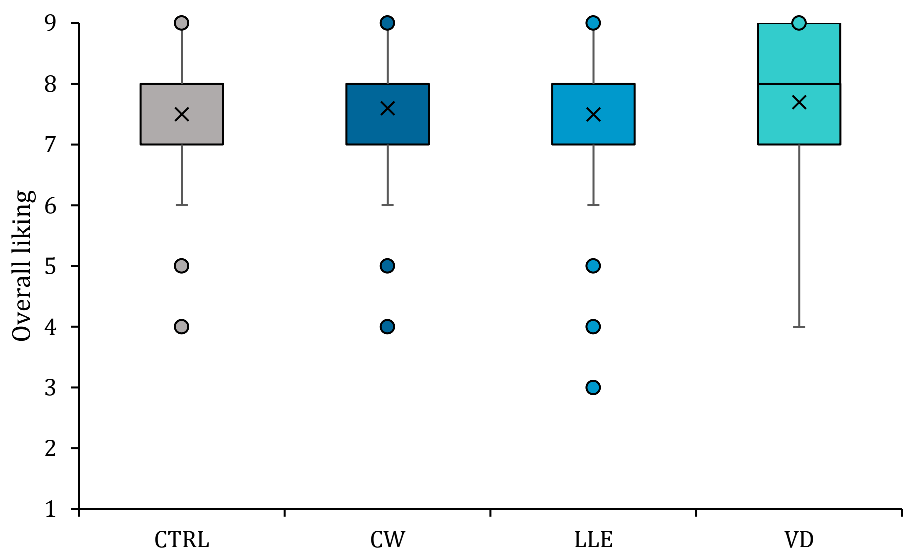

Figure 4 – Box and whisker plots of the distribution of overall liking ratings (9-point hedonic scale, from 1 to 9), by the consumer panel (n=70) of fish samples from different dietary treatments.

4. Discussion

The goal of this study was to evaluate the possibility of using aromas derived from sardine cooking wastewaters, obtained under a circular economy approach, as potential feed intake modulators for European seabass. Additionally, the study aimed to assess the impact of these aromas on the overall flesh quality of farmed fish. The ingredients used in this work were mostly rich in alcohols and aldehydes, which derive from the breakdown of sardine oil during the steaming process (Ferraro et al., 2013). To the best of the authors' knowledge, this represents the first study addressing aroma-rich extracts resulting from fish cooking wastewaters as potential feed intake modulators in a long-term trial.

The supplemented diets did not result in any discernible differences in terms of fish weight gain, as all treatments achieved similar final weights. This outcome was observed despite significant differences in VFI, indicating a change in the FCR. Fish fed the LLE diet displayed the highest VFI, but also showed the highest FCR and the lowest PER. These differences were statistically significant when compared to the CW group. Additionally, we observed a decrease in circulating glucose in the LLE group 6 h after feeding, which could potentially contribute to lower energy levels in comparison to the other groups. This reduction appears to trigger a cascade of signals that lead to an increase in *npy* levels in the hypothalamus, as evidenced by the positive correlation between VFI and *npy* expression in the hypothalamus. Consequently, this initiates an orexigenic response, stimulating appetite, and promoting food intake. Overall, these results suggest that the LLE diet might be influencing intake through a homeostatic regulatory mechanism. It is likely that the metabolism has been impaired, leading to suboptimal nutrient utilization. One plausible explanation could be that fish are less efficient at utilising lipids from the LLE diet, or that this diet hinders lipid digestion, causing fish to favour proteins as an energy source. This preference for proteins might explain the worse FCR and PER observed. To compensate these effects, fish increase their overall feed intake to maintain nutrient and energy homeostasis, ultimately leading to comparable nutrient gain and weight gain. Interestingly, the LLE group displayed the highest concentration of glucose in the liver, even though there were no statistically significant differences in liver glycogen. This suggests that the glycogen in the liver is being converted into glucose, which is then released into the bloodstream, potentially as an attempt to counteract the low plasmatic levels of glucose. The LLE diet also resulted in the highest levels of triglycerides in the liver, despite no significant impact on the total lipids content due to dietary treatments. This suggests a potential disturbance in lipid metabolism, raising the possibility that triglycerides may not be efficiently used as energy sources and are instead deposited in the liver. However, it is worth noting that the nutrient gain values reported in this study fall within the range previously documented in the literature for this species (Batista et al., 2020; Pereira et al., 2022b).

The LLE-A was derived using soybean oil, and previous research reported that soybean oil might lead to an increased FCR, possibly due to an unbalanced fatty acid profile when compared to fish oil (Trushenski et al., 2013; Emre et al., 2016). It is also plausible than certain compounds present in the LLE-A extract may be influencing fish metabolism, either directly or indirectly. For example, these compounds might be affecting microbiota diversity, which, in turn, can impact fish nutrient digestibility and utilization. In future research, it might be worthwhile to explore alternative oil-based vehicles for extraction, such as microalgae oil. Microalgae oil has been reported to possess valuable properties for aquafeeds, including high levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as antioxidants (Shah et al., 2018; Nagappan et al., 2021), which could open up the possibility of obtaining an extract that not only enhances VFI but also provides welfare-promoting properties for fish. However, the increase in the intake may also be a result of a hedonic regulation or, most likely, a combination of both homeostatic and hedonic influences. It has been reported that fish have high sensitivity to fatty acids (Soengas, 2014). While long and medium chain fatty acids appear to reduce feed intake (Velasco et al., 2020; Feng et al., 2022), short chain fatty acids may have the opposite impact (Zhang et al., 2020). Pentanoic acid and hexanoic acid, which are short-chain fatty acids, were exclusively found in the LLE-A extract, and these compounds might account for the slight increase on VFI. Additionally, aliphatic acids have previously been identified, through electrophysiological recordings, as having stimulatory properties in Japanese eel, Atlantic salmon and common carp. Notably, aliphatic acids with 3-5 carbons were found to be particularly effective in the latter species (Marui and Caprio, 1992; Kasumyan and Døving, 2003). Hexanal has previously been described as having a “fishy” odour (Giri et al., 2010), and LLE-A happens to contain the highest levels of this compound. In contrast, the CW-A presented high levels of pentanal and 2,4-heptadienal, which have been associated with a “rubber” and “rancid” odour (Venkateshwarlu et al., 2004). These compounds can function as off-flavours and discourage feed intake. The impact of aldehydes on fish taste response has received little attention. Jones (1990) reported that rainbow trout exhibited minimal to no response to aldehydes with 3-5 carbons. This study also found that *n*-hexanol and *n*-octanol were the most palatable alcohols. Both were found in LLE-A and VD-A. However, these compounds were not detected in the extract CW-A, and this diet also resulted in the lowest VFI; these two evidences could possibly be connected. However, it is worth noting that Jones (1990) performed the study using cotton pellets soaked in the compound solution rather than practical diets. The possibility of synergic or antagonistic effects within a complex mixture such as practical diets, which contain a multitude of nutrients beyond those found in the aromatic extracts, cannot be discarded. Additionally, fish fed CW displayed the lowest FCR and highest PER. Therefore, this might suggest that fish on this diet can efficiently utilize nutrients to meet their energetic needs, and since hedonic regulation is not triggered, the feed intake remains unaltered.

Despite variations in VFI observed among the supplemented diets, there were no significant differences found in the mRNA abundance of the neuropeptides involved in the regulation of feed intake. This lack of significant differences might be attributed to a potential habituation effect to the diets (Basto et al., 2021), as the analysis was performed after an 18-week feeding trial. Although a*grp* levels were slightly elevated in the hypothalamus for the animals on supplemented diets, this increase was not enough to lead to a significant increase in their feed intake compared to the control group. In addition, the orexigenic *npy* was slightly upregulated in the hypothalamus for the LLE diet compared to the CW diet. This partially explains the increased VFI observed in fish fed on the LLE diet. This relationship is further supported by the positive correlation found between *npy* expression and VFI. The neuropeptide responses in the telencephalon were slightly different compared to those in the hypothalamus, with less variation observed in response to diets. This suggests that the diet had a comparatively lesser impact on the telencephalon. A similar pattern was found by Comesaña et al. (2018a) when studying the effect of valine and leucine on the feed intake of rainbow trout. They found that feed intake was more closely associated with neuropeptide expression in the hypothalamus than in the telencephalon, highlighting the importance of the hypothalamus in regulating feed intake. It should be noted that *pomca* was not detected in European sea bass telencephalon by RT-qPCR, confirming previous observations by Basto et al. (2021).

Skin and fillet colour are very important during the purchase decision, as they are among the first sensory attributes consumers notice. When diets are rich in plant-based feed ingredients, there is a concern that the skin may appear darker or duller coloured, which consumers often associate with a product that is less fresh (Pleić et al., 2022). In this study, the dietary treatments did not influence skin nor fillet colour. This was expected, since the aromatic extracts did not possess significative pigmentation. The instrumental colour parameters observed in this study are in line with previous literature reports on European seabass fed diets rich in plant-based ingredients (Bonvini et al., 2018).

Additionally, the textural properties of the muscle play a crucial role in consumer acceptance of the product. These properties can be affected by both nutrient deposition in the muscle and the animals' growth performance, making them susceptible to dietary manipulations. In this work, only hardness was significantly affected by diets, with the control displaying a higher hardness than the LLE diet. However, this difference was not perceived by the consumer panel, as all samples were generally rated as *juicy*, *tender* and *easy to flake*. This discrepancy between instrumental and panel analysis can be attributed to the fact that instrumental analysis is conducted on raw fish, while sensory evaluation is based on cooked fish (Kotzamanis et al., 2020), making any minor differences undiscernible by consumers. Changes in fillet texture can be a attributed to factors like muscle cell density and fibre cross-sectional area (Matos et al., 2012). Conducting a histological assessment of the muscle could possibly shed some light on the reason behind the observed differences in this parameter. It should also be mentioned that the use of soybean oil as an extract medium might be a contributing factor. Previous studies reported a small decrease in fillet firmness in seabream when soybean oil was included in their diets (Izquierdo et al., 2003; Izquierdo et al., 2005).

Regarding odour and flavour, no differences were detected by the consumer panel. This suggests that the aromas did not accumulate in the fish fillet and, as a result, did not impact the flavour and odour typically associated with European seabass. This is in agreement with the work of Iniesta et al. (2022), who reported that the dietary inclusion of essential oils, also rich in aroma compounds, did not affect gilthead seabream flavour as evaluated by a sensory panel. A similar situation occurred in pork meat, where the inclusion of essential oils from oregano, rosemary, garlic or ginger (all rich in aroma and flavour compounds) in pig diets did not lead to any noticeable differences in meat flavour or odour (Janz et al., 2007).

Moreover, consumer liking of all samples was high, with a median rating of 8, and all dietary treatments received over 90% positive (6 – "slightly like or above”) scores. This indicates that all tested diets produced fish of high quality for consumption, as perceived by consumers.

5. Conclusions

The diet LLE has resulted in a slight increase on fish voluntary feed intake (VFI) but has also elevated the feed conversion ratio (FCR). The increase in intake was correlated with a higher expression of neuropeptide y in the hypothalamus. Moreover, these aromas did not affect other growth performance indicators, nor did they compromise the sensory profile of fish muscle. Overall, while this innovative approach holds promise, it is essential to conduct studies to determine the optimal inclusion levels of these aromas in diets through dosage evaluations to maximize their effectiveness.

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Data availability

Data will be made available on request.

Declarations of interest

None.

References

Ares, G., Bruzzone, F., Vidal, L., Cadena, R.S., Giménez, A., Pineau, B., Hunter, D.C., Paisley, A.G., Jaeger, S.R., 2014. Evaluation of a rating-based variant of check-all-that-

apply questions: Rate-all-that-apply (RATA). Food Qual. Prefer. 36, 87-95. https://doi.org/10.1016/j.foodqual.2014.03.006.
Baião, L.F., Rocha, C., Lima, R.C., Valente, L.M.P., Cunha, L.M., 2022. Development of a Rate-All-That-Apply (RATA) ballot for sensory profiling of sea urchin (*Paracentrotus lividus*) gonads. Food Res. Int. 153, 110976. https://doi.org/10.1016/j.foodres.2022.110976.
Basto, A., Valente, L.M.P., Conde-Sieira, M., Soengas, J.L., 2021. Central regulation of food intake is not affected by inclusion of defatted *Tenebrio molitor* larvae meal in diets for European sea bass (*Dicentrarchus labrax*). Aquaculture 544, 737088. https://doi.org/10.1016/j.aquaculture.2021.737088.
Basto, A., Valente, L.M.P., Soengas, J.L., Conde-Sieira, M., 2022. Partial and total fishmeal replacement by defatted *Tenebrio molitor* larvae meal do not alter short- and mid-term regulation of food intake in European sea bass (*Dicentrarchus labrax*). Aquaculture 560, 738604. https://doi.org/10.1016/j.aquaculture.2022.738604.
Batista, S., Pereira, R., Oliveira, B., Baião, L.F., Jessen, F., Tulli, F., Messina, M., Silva, J.L., Abreu, H., Valente, L.M.P., 2020. Exploring the potential of seaweed *Gracilaria gracilis* and microalga *Nannochloropsis oceanica*, single or blended, as natural dietary ingredients for European seabass *Dicentrarchus labrax*. J. Appl. Phycol. 32, 2041-2059. https://doi.org/10.1007/s10811-020-02118-z.
Bonvini, E., Parma, L., Badiani, A., Fontanillas, R., Gatta, P.P., Sirri, F., Nannoni, E., Bonaldo, A., 2018. Integrated study on production performance and quality traits of European sea bass (*Dicentrarchus labrax*) fed high plant protein diets. Aquaculture 484, 126-132. https://doi.org/10.1016/j.aquaculture.2017.10.041.
Chen, M., Chen, X., Nsor-Atindana, J., Masamba, K.G., Ma, J., Zhong, F., 2017. Optimization of key aroma compounds for dog food attractant. Anim. Feed Sci. Technol. 225, 173-181. https://doi.org/10.1016/j.anifeedsci.2016.12.005.
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*). Aquac. Nutr. 19, 773-784. https://doi.org/10.1111/anu.12024.
Comesaña, S., Velasco, C., Ceinos, R.M., López-Patiño, M.A., Míguez, J.M., Morais, S., Soengas, J.L., 2018a. Evidence for the presence in rainbow trout brain of amino acid-sensing systems involved in the control of food intake. Am. J. Physiol. Regul. Integr. Comp. Physiol. 314, R201-R215. https://doi.org/10.1152/ajpregu.00283.2017.
Comesaña, S., Velasco, C., Conde-Sieira, M., Míguez, J.M., Soengas, J.L., Morais, S., 2018b. Feeding stimulation ability and central effects of intraperitoneal treatment of L-leucine, L-valine, and L-proline on amino acid sensing systems in rainbow trout:

Implication in food intake control. Front. Physiol. 9, 1209. https://doi.org/10.3389/fphys.2018.01209.

Dauda, A.B., Ajadi, A., Tola-Fabunmi, A.S., Akinwole, A.O., 2019. Waste production in aquaculture: sources, components and managements in different culture systems. Aquac. Fish. 4, 81-88. https://doi.org/10.1016/j.aaf.2018.10.002.

Delgado, M.J., Cerdá-Reverter, J.M., Soengas, J.L., 2017. Hypothalamic integration of metabolic, endocrine, and circadian signals in fish: involvement in the control of food intake. Front. Neurosci. 11, 354. https://doi.org/10.3389/fnins.2017.00354.

Dias, J., Gomes, E.F., Kaushik, S., 1997. Improvement of feed intake through supplementation with an attractant mix in European bass fed plant-protein rich diets. Aquat. Living Resour. 10. https://doi.org/10.1051/alr:1997043.

Díaz-Rúa, A., Chivite, M., Comesaña, S., Conde-Sieira, M., Soengas, J.L., 2022. The opioid system in rainbow trout telencephalon is probably involved in the hedonic regulation of food intake. Front. Physiol. 13. https://doi.org/10.3389/fphys.2022.800218.

Emre, Y., Kurtoğlu, A., Emre, N., Güroy, B., Güroy, D., 2016. Effect of replacing dietary fish oil with soybean oil on growth performance, fatty acid composition and haematological parameters of juvenile meagre, *Argyrosomus regius*. Aquac. Res. 47, 2256-2265. https://doi.org/10.1111/are.12677.

Feng, H., Peng, D., Liang, X.-F., Li, J., Luo, H., Tang, S., Chai, F., 2022. Intracerebroventricular injection with octanoic acid activates hypothalamic fatty acid sensing systems and regulates appetite in Chinese perch *Siniperca chuatsi*. Fish Sci. 88, 83-90. https://doi.org/10.1007/s12562-021-01570-1.

Ferraro, V., Carvalho, A.P., Piccirillo, C., Santos, M.M., L. Castro, P.M., E. Pintado, M., 2013. Extraction of high added value biological compounds from sardine, sardine-type fish and mackerel canning residues — A review. Mater. Sci. Eng. C 33, 3111-3120. https://doi.org/10.1016/j.msec.2013.04.003.

Fountoulaki, E., Vasilaki, A., Hurtado, R., Grigorakis, K., Karacostas, I., Nengas, I., Rigos, G., Kotzamanis, Y., Venou, B., Alexis, M.N., 2009. Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects on growth performance, flesh quality and fillet fatty acid profile: Recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. Aquaculture 289, 317-326. https://doi.org/10.1016/j.aquaculture.2009.01.023.

Ganeko, N., Shoda, M., Hirohara, I., Bhadra, A., Ishida, T., Matsuda, H., Takamura, H., Matoba, T., 2008. Analysis of volatile flavor compounds of sardine (*Sardinops melanostica*) by Solid Phase Microextraction. J. Food Sci. 73, S83-S88. https://doi.org/10.1111/j.1750-3841.2007.00608.x.

Giri, A., Osako, K., Ohshima, T., 2010. Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. Food Chem. 120, 621-631. https://doi.org/10.1016/j.foodchem.2009.10.036.

Hossain, M.S., Koshio, S., Kestemont, P., 2020. Recent advances of nucleotide nutrition research in aquaculture: a review. Rev. Aquac. 12, 1028-1053. https://doi.org/10.1111/raq.12370.

Iniesta, C., Zapata, E., Firmino, J.P., Peñaranda, I., Egea, M., Linares, M.B., Garrido, M.D., 2022. Effect of the dietary supplementation based on essential oils on the quality of gilthead seabream. Aquac. Res. 53, 2567-2574. https://doi.org/10.1111/are.15796.

Izquierdo, M.S., Montero, D., Robaina, L., Caballero, M.J., Rosenlund, G., Ginés, R., 2005. Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture 250, 431-444. https://doi.org/10.1016/j.aquaculture.2004.12.001.

Izquierdo, M.S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L., Rosenlund, G., 2003. Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. Aquac. Nutr. 9, 397-407. https://doi.org/10.1046/j.1365-2095.2003.00270.x.

Janz, J.A.M., Morel, P.C.H., Wilkinson, B.H.P., Purchas, R.W., 2007. Preliminary investigation of the effects of low-level dietary inclusion of fragrant essential oils and oleoresins on pig performance and pork quality. Meat Sci. 75, 350-355. https://doi.org/10.1016/j.meatsci.2006.06.027.

Jones, K.A., 1990. Chemical requirements of feeding in rainbow trout, *Oncorhynchus mykiss* (Walbaum); palatability studies on amino acids, amides, amines, alcohols, aldehydes, saccharides, and other compounds. J. Fish. Biol. 37, 413-423. https://doi.org/10.1111/j.1095-8649.1990.tb05872.x.

Kasumyan, A.O., Døving, K.B., 2003. Taste preferences in fishes. Fish Fish. 4, 289-347. https://doi.org/10.1046/j.1467-2979.2003.00121.x.

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.

Kokou, F., Fountoulaki, E., 2018. Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. Aquaculture 495, 295-310. https://doi.org/10.1016/j.aquaculture.2018.06.003.

Kotzamanis, Y., Kumar, V., Tsironi, T., Grigorakis, K., Ilia, V., Vatsos, I., Brezas, A., van Eys, J., Gisbert, E., 2020. Taurine supplementation in high-soy diets affects fillet quality of European sea bass (*Dicentrarchus labrax*). Aquaculture 520, 734655. https://doi.org/10.1016/j.aquaculture.2019.734655.

Lim, L.-S., Chor, W.-K., Tuzan, A.D., Shapawi, R., Kawamura, G., 2016. Betaine is a feed enhancer for juvenile grouper (*Epinephelus fuscoguttatus*) as determined behaviourally. J. Appl. Anim. Res. 44, 415-418. https://doi.org/10.1080/09712119.2015.1091329.

Lin, X., Volkoff, H., Narnaware, Y., Bernier, N.J., Peyon, P., Peter, R.E., 2000. Brain regulation of feeding behavior and food intake in fish. Comp. Biochem. Phys. A 126, 415-434. https://doi.org/10.1016/S1095-6433(00)00230-0.

Luo, J.-B., Feng, L., Jiang, W.-D., Liu, Y., Wu, P., Jiang, J., Kuang, S.-Y., Tang, L., Tang, W.-N., Zhang, Y.-A., Zhou, X.-Q., 2017. Physical and flavor characteristics, fatty acid profile, antioxidant status and nrf2-dependent antioxidant enzyme gene expression changes in young grass carp (*Ctenopharyngodon idella*) fillets fed dietary valine. PLOS ONE 12, e0169270. https://doi.org/10.1371/journal.pone.0169270.

Ma, X.-Z., Feng, L., Wu, P., Liu, Y., Kuang, S.-Y., Tang, L., Zhou, X.-Q., Jiang, W.-D., 2020. Enhancement of flavor and healthcare substances, mouthfeel parameters and collagen synthesis in the muscle of on-growing grass carp (*Ctenopharyngodon idella*) fed with graded levels of glutamine. Aquaculture 528, 735486. https://doi.org/10.1016/j.aquaculture.2020.735486.

Mackie, A.M., Adron, J.W., Grant, P.T., 1980. Chemical nature of feeding stimulants for the juvenile Dover sole, *Solea solea* (L.). J. Fish. Biol. 16, 701-708. https://doi.org/10.1111/j.1095-8649.1980.tb03749.x.

Makol, A., Torrecillas, S., Vaquero, A., Rincon, L., Ginés, R., Izquierdo, M., 2013. Deposition of conjugated linoleic acid in market size sea bass (*Dicentrarchus labrax*) and its effects on performance, composition and fillet sensory and texture attributes. Aquac. Nutr. 19. https://doi.org/10.1111/anu.12025.

Marui, T., Caprio, J., 1992. Teleost gustation, In: Hara, T.J. (Ed.), Fish Chemoreception, Chapman & Hall, Winnipeg, Canada, pp. 171-198.

Matos, E., Gonçalves, A., Bandarra, N., Colen, R., Nunes, M.L., Valente, L.M.P., Dinis, M.T., Dias, J., 2012. Plant proteins and vegetable oil do not have detrimental effects on post-mortem muscle instrumental texture, sensory properties and nutritional value of gilthead seabream. Aquaculture 358-359, 205-212. https://doi.org/10.1016/j.aquaculture.2012.07.009.

Mendes, R., Gonçalves, A., 2008. Effect of soluble CO_2 stabilisation and vacuum packaging in the shelf life of farmed sea bream and sea bass fillets. Int. J. Food Sci. Tech. 43, 1678-1687. https://doi.org/10.1111/j.1365-2621.2008.01737.x.

Montero, D., Robaina, L., Caballero, M.J., Ginés, R., Izquierdo, M.S., 2005. Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: A time-course study on the effect of a re-feeding period with a 100% fish oil diet. Aquaculture 248, 121-134. https://doi.org/10.1016/j.aquaculture.2005.03.003.

Nagappan, S., Das, P., AbdulQuadir, M., Thaher, M., Khan, S., Mahata, C., Al-Jabri, H., Vatland, A.K., Kumar, G., 2021. Potential of microalgae as a sustainable feed ingredient for aquaculture. J. Biotechnol. 341, 1-20. https://doi.org/10.1016/j.jbiotec.2021.09.003.

National Research Council, 2011. Nutrient requirements of fish and shrimp. The National Academies Press, Washington, DC.

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, 551-563. https://doi.org/10.1038/s41586-021-03308-6.

Pereira, M.J., Grosjean, O., Pintado, M., Brazinha, C., Crespo, J., 2022a. Clean technologies for production of valuable fractions from sardine cooking wastewaters: an integrated process of flocculation and reverse osmosis. Clean Technol. 4, 276-295. https://doi.org/10.3390/cleantechnol4020016.

Pereira, R., Costa, M., Velasco, C., Cunha, L.M., Lima, R.C., Baião, L.F., Batista, S., Marques, A., Sá, T., Campos, D.A., Pereira, M., Jesus, D., Fernández-Boo, S., Costas, B., Pintado, M., Valente, L.M.P., 2022b. Comparative analysis between synthetic vitamin E and natural antioxidant sources from tomato, carrot and coriander in diets for market-sized *Dicentrarchus labrax*. Antioxidants 11, 636. https://doi.org/10.3390/antiox11040636.

Peryam, D.R., Pilgrim, F.J., 1957. Hedonic scale method of measuring food preferences. Food Technol. 11, 9-14.

Pleić, I.L., Bušelić, I., Messina, M., Hrabar, J., Žuvić, L., Talijančić, I., Žužul, I., Pavelin, T., Anđelić, I., Pleadin, J., Puizina, J., Grubišić, L., Tibaldi, E., Šegvić-Bubić, T., 2022. A plant-based diet supplemented with *Hermetia illucens* alone or in combination with poultry by-product meal: one step closer to sustainable aquafeeds for European seabass. J. Animal Sci. Biotechnol. 13, 77. https://doi.org/10.1186/s40104-022-00725-z.

Prem, R., Tewari, V.K., 2020. Development of human-powered fish feeding machine for freshwater aquaculture farms of developing countries. Aquac. Eng. 88, 102028. https://doi.org/10.1016/j.aquaeng.2019.102028.

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, M.J., Sá, T., Brazinha, C., Pintado, M., Valente, L.M.P., Velasco, C., 2024. Production of aroma-rich extracts from sardine cooking wastewaters: Exploring their potential for modulating feed intake in European seabass. Waste Biomass Valoriz. [Accepted for publication].

Schlüter, S., Steinhart, H., Schwarz, F.J., Kirchgessner, M., 1999. Changes in the odorants of boiled carp fillet (*Cyprinus carpio* L.) as affected by increasing methionine levels in feed. J. Agric. Food Chem. 47, 5146-5150. https://doi.org/10.1021/jf9902604.

Shah, M.R., Lutzu, G.A., Alam, A., Sarker, P., Kabir Chowdhury, M.A., Parsaeimehr, A., Liang, Y., Daroch, M., 2018. Microalgae in aquafeeds for a sustainable aquaculture industry. J. Appl. Phycol. 30, 197-213. https://doi.org/10.1007/s10811-017-1234-z.

Soengas, J.L., 2014. Contribution of glucose- and fatty acid sensing systems to the regulation of food intake in fish. A review. Gen. Comp. Endocrinol. 205, 36-48. https://doi.org/10.1016/j.ygcen.2014.01.015.

Soengas, J.L., 2021. Integration of nutrient sensing in fish hypothalamus. Front. Neurosci. 15, 653928. https://doi.org/10.3389/fnins.2021.653928.

Soengas, J.L., Cerdá-Reverter, J.M., Delgado, M.J., 2018. Central regulation of food intake in fish: an evolutionary perspective. J. Mol. Endocrinol. 60, R171-R199. https://doi.org/10.1530/jme-17-0320.

Takács, S., Musso, A.E., Gries, R., Rozenberg, E., Borden, J.H., Brodie, B., Gries, G., 2018. New food baits for trapping house mice, black rats and brown rats. Appl. Anim. Behav. Sci. 200, 130-135. https://doi.org/10.1016/j.applanim.2017.11.011.

Torrecillas, S., Robaina, L., Caballero, M.J., Montero, D., Calandra, G., Mompel, D., Karalazos, V., Kaushik, S., Izquierdo, M.S., 2017. Combined replacement of fishmeal and fish oil in European sea bass (Dicentrarchus labrax): Production performance, tissue composition and liver morphology. Aquaculture 474, 101-112. https://doi.org/https://doi.org/10.1016/j.aquaculture.2017.03.031.

Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbø, R., Hemre, G.I., Fontanillas, R., Nordgarden, U., Hevrøy, E.M., Olsvik, P., Berntssen, M.H.G., 2008. Novel production of Atlantic salmon (*Salmo salar*) protein based on combined

replacement of fish meal and fish oil with plant meal and vegetable oil blends. Aquaculture 285, 193-200. https://doi.org/10.1016/j.aquaculture.2008.08.025.
Trushenski, J., Mulligan, B., Jirsa, D., Drawbridge, M., 2013. Sparing fish oil with soybean oil in feeds for white seabass: effects of inclusion rate and soybean oil composition. N. Am. J. Aquac. 75, 305-315. https://doi.org/10.1080/15222055.2012.720650.
Utne-Palm, A.C., Bogevik, A.S., Humborstad, O.-B., Aspevik, T., Pennington, M., Løkkeborg, S., 2020. Feeding response of Atlantic cod (*Gadus morhua*) to attractants made from by-products from the fishing industry. Fish. Res. 227, 105535. https://doi.org/10.1016/j.fishres.2020.105535.
Velasco, C., Conde-Sieira, M., Comesaña, S., Chivite, M., Díaz-Rúa, A., Míguez, J.M., Soengas, J.L., 2020. The long-chain fatty acid receptors FFA1 and FFA4 are involved in food intake regulation in fish brain. J. Exp. Biol. 223, jeb227330. https://doi.org/10.1242/jeb.227330.
Velasco, C., Conde-Sieira, M., Comesaña, S., Chivite, M., Míguez, J.M., Soengas, J.L., 2021. Role of the G protein-coupled receptors GPR84 and GPR119 in the central regulation of food intake in rainbow trout. J. Exp. Biol. 224, jeb242360. https://doi.org/10.1242/jeb.242360.
Venkateshwarlu, G., Let, M.B., Meyer, A.S., Jacobsen, C., 2004. Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. J. Agric. Food Chem. 52, 311-317. https://doi.org/10.1021/jf034833v.
Zhang, H., Ding, Q., Wang, A., Liu, Y., Teame, T., Ran, C., Yang, Y., He, S., Zhou, W., Olsen, R.E., Zhang, Z., Zhou, Z., 2020. Effects of dietary sodium acetate on food intake, weight gain, intestinal digestive enzyme activities, energy metabolism and gut microbiota in cultured fish: Zebrafish as a model. Aquaculture 523, 735188. https://doi.org/10.1016/j.aquaculture.2020.735188.

5. CONSUMER PERCEPTION OF AQUACULTURE PRODUCTS

5.1. Emerging themes on the perception of wild and farmed fish

This work is in preparation for submission to *Reviews in Aquaculture*, as follows.

Emerging themes on the perception of wild and farmed fish

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Abstract

Despite the growth of aquaculture over the past years, consumers still have a more positive perception of wild fish products. Following the PRISMA methodology, a literature review was performed to investigate this issue and the reasons behind such consumer behaviour. The search query was "consumer AND fish AND aquaculture", and the assessed databases were Scopus and Web of Science. After removing duplicates and irrelevant articles, 186 reports were included in the review. The reviewed papers verify that wild fish is still a reference for consumers. Farmed fish are generally considered safer, more controlled, and less affected by water pollution. It is also perceived as less expensive and more readily available. However, wild fish are perceived as having fewer chemicals from feeding, a better quality (associated with higher price), being more natural, environmentally friendly, and promoting better fish welfare. Overall, the review also highlighted the lack of consumer knowledge about matters affecting aquaculture and fisheries. Other aspects also stood out as significant for consumers, including the country of origin of products and the growing focus on ethical aspects of products. The most relevant demographic variables regarding the preference for wild and farmed fish were also assessed, and in general, wild fish is favoured by older, traditionalist consumers. Finally, some considerations for improving the perception of farmed fish are suggested.

Keywords: Fish acceptance; Aquaculture; Catches; Consumer perception.

1. Introduction

The increase in fish demand that has led to a worldwide expansion of fishing fleets and higher fish capture efficiency has contributed to overfishing and the risk of depletion of some natural fish stocks [1]. The proportion of overexploited, depleted and recovering stocks has remained relatively stable during the last 10-15 years. Data from 2019 showed that 35.4% of fish stocks were estimated to be fished at a biologically unsustainable level and therefore overfished, whereas fully fished stocks accounted for 57.3% and underfished stocks 7.2% [2]. Fish is an important protein source, with well-established health and nutrition benefits [3,4], cultural significance in many regions around the world [5-7] and important environmental advantages compared to other animal-based protein sources [8]. The countries where consumption is highest include Iceland, the Faroe Islands and Maldives, which consume over 80 kg of aquatic foods per capita per year. In Europe, apart from the aforementioned countries, Portugal and Norway are also notably high seafood consumers (56 and 55 kg per capita per year, respectively) [9]; in Asia, South Korea and Malaysia display high consumption levels as well (above 50 kg per capita per year) [2]. In response to these drivers, farmed fish, i.e., fish obtained through aquaculture production systems, has become a valuable alternative. Aquaculture is one of the agri-food sectors with the fastest growth over the past years [2]. The expansion in global trades, rising unavailability of wild fish, affordable pricing and global urbanization have all contributed to aquaculture development and growth [10-12]. Currently, over 425 species, including fish, shellfish, and seaweeds, can be farmed [13]. In particular, finfish comprise about 50% of the total aquaculture production (in weight) [14].

Despite these trends, farmed fish sales are still below expected, either due to a low consumer pressure or due to production constraints. In 2020, in the European Union, aquaculture production was not able to surpass wild-caught fish, neither in weight nor in volume, according to data from the European Market Observatory for Fisheries and Aquaculture Products [9]. Catches comprised EUR 5.85 billion (3.59 million tonnes) of all seafood production in the European Union (EU), contrasting with EUR 4.17 billion (1.13 million tonnes) for aquaculture production [9]. Moreover, when analysing the European Union per capita apparent consumption (apparent consumption = imports + aquaculture production + catches – exports) of aquaculture and fishery products, it is clear that catches still represent a significant 70% of the total consumption, and this has been relatively steady over the last decade [9]. A survey from the Eurobarometer, about consumer habits regarding fishery and aquaculture products [15], has also revealed that 32% of EU consumers still prefer wild-caught products given the choice, with only 7% claiming to prefer farmed products. These data hint at a possible negative image of aquaculture products. The reasons for this preference should be deeply studied, as the success of aquaculture business also depends on its perceived image and consumer acceptance. It is important to understand if this phenomenon is product-dependent and how it varies due to geographical, cultural, and temporal background. Only then can one make suggestions on improving the image of aquaculture products.

Therefore, we performed a systematic literature review of studies assessing consumer perception of aquaculture products to extract this information. Moreover, considering the significance of fish as a protein source, we have decided to focus on works analysing finfish products, excluding other types of seafood (e.g., algae, shellfish, crustaceans). With the obtained results, suggestions will be made to improve aquaculture's image among consumers.

2. Methodology

A systematic literature review was employed to identify the major research issues and emerging themes in consumer perceptions towards aquaculture. The research was conducted using Scopus and Clarivate's Web of Science database for the retrieval of literature reports, in line with the recommendations from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [16]. Works published until 2023 were considered and the databases were last consulted on March 2024.

The expression "Consumer AND (aquaculture OR wild OR farmed) AND fish" was set as a search query in this review. Results from both databases were imported into the Endnote TM software (Clarivate TM, version 20.5 Bld 16860). Duplicates were removed using this software, and a first screening was performed to select the studies to be included in this review. Reports had to be related to the consumer perceptions and their opinions towards aquaculture. Studies with a particular focus on non-finfish species, such as crustaceans, shellfish, seaweeds, or others, were excluded, as we aimed to focus on finfish. The full text had to be available, and only original research articles were considered. Finally, the text had to be in English. Figure 1 shows the flow diagram that represents the procedure followed during this review.

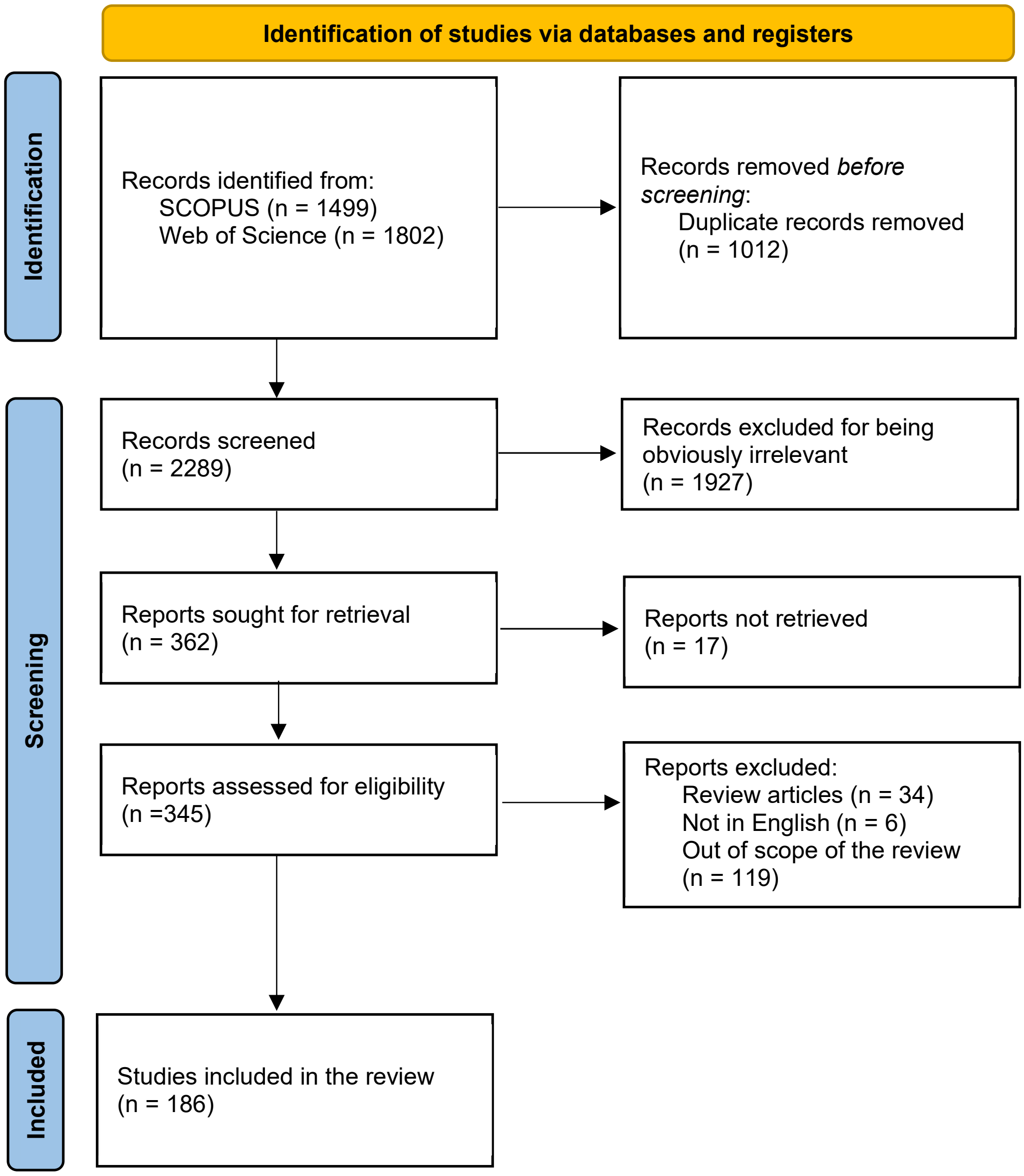

Figure 1 - Flow diagram of the procedure applied during the review work, using the search query "Consumer AND (aquaculture OR wild OR farmed) AND fish", following the PRISMA methodology.

3. Results and Discussion

3.1. Overview of identified studies

A total of 3,301 reports were retrieved from the databases, with 1,012 duplicates being excluded (Figure 1). This resulted in 2,289 screened reports, of which 1,927 were excluded due to lack of relevance. Of the 362 reports sought for retrieval, 17 were not retrieved. Then, 34 articles were excluded due to not being original research articles, six due to being in a language other than English, and 119 from being out of the scope of this work. Finally, 186 studies were included in this review. A table describing the 186 studies is provided as Supplementary Material.

Generally, the reports regarding consumer perceptions towards aquaculture and farmed fish have increased over the years (Figure 2).

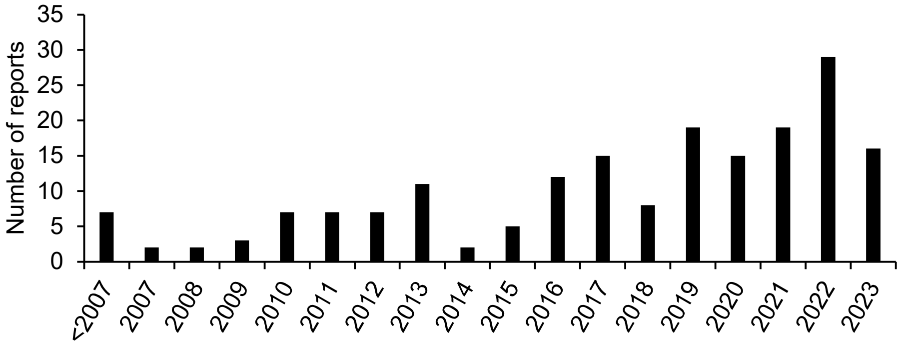

Figure 2 – The number of published studies identified in this work that focus on consumer perceptions of wild and farmed fish over the years.

Regarding the geographic focus of the studies, the United States, Germany, and Spain were the most frequently studied countries. A significant fraction of studies have also performed cross-cultural comparisons (Figure 3).

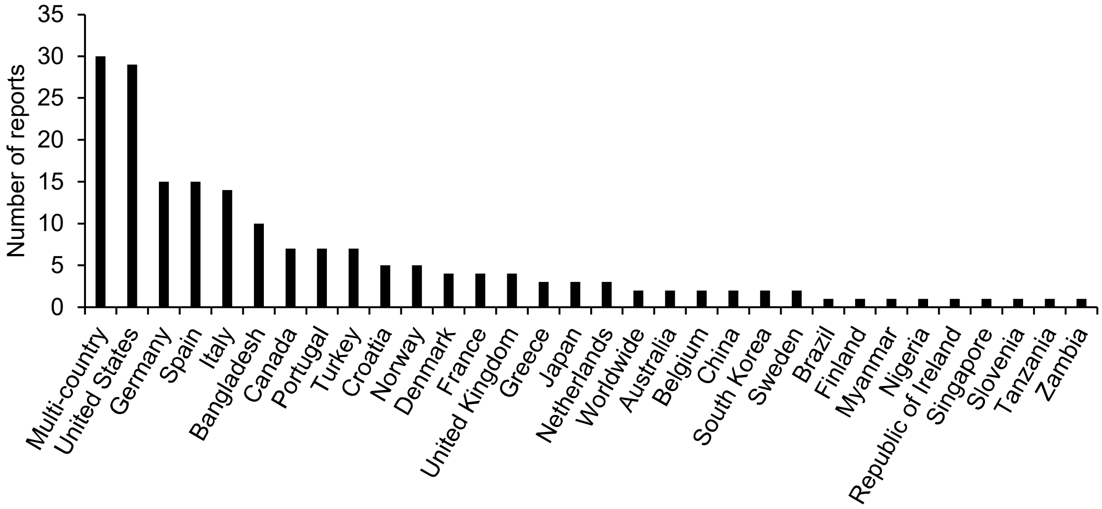

Figure 3 – Geographical focus of the surveyed studies regarding consumer perception of wild and farmed fish.

The most frequently used research method to evaluate consumers' perceptions and behaviours regarding wild and farmed fish were surveys (126 reports), followed by choice experiments (43) and focus groups (13). Additionally, the most common method of conducting surveys for these studies was face-to-face (83 reports), followed by online (74) methods, despite some studies still resourcing to mail (8) and over-the-phone (6) interactions to communicate with participants.

The following sections will focus on the emerging themes that were identified from the analysis of the identified works.

3.2. Perceptions regarding safety and control

The concept of safety involves processing considerations, microbiological contaminations, and the presence of chemicals, either from feeding, environmental sources, or cross-contaminations. Considering that fish is frequently bought in its raw state, safety becomes a significant aspect at purchasing moment, due to its perishability [17,18].

One of the main reasons pointed out by consumers for their worse perception of farmed fish products relies on their belief that farmers tend to overuse “chemicals” when growing fish [19-37]. These “chemicals”, to the consumers’ eyes, can be undefined [19,20,23,25,26,29-31,33,35,37], but also include antibiotics [22,23,27,28,34,36,38], hormones [20,23,34,35], preservatives [39] or pigments [24]. No association was found between the belief in the presence of chemicals in farmed fish and any specific geographical region or species; this notion is disseminated in Europe, North America, and Asia and applies to all species. These chemicals are perceived as being harmful to human health and the environment and thus rejected by consumers [20]. Indeed, uncontrolled antibiotic usage can promote antibiotic resistance dissemination within the environment, with worrying consequences for their therapeutic effectiveness [40]. Therefore, an effort has been made to regulate the usage of drugs and antibiotics in aquaculture, namely in the European Union (Regulation 2019/4 on the manufacture, placing on the market and use of medicated feed [41]) and the USA (FDA regulation on approved drugs for use in seafoods [42]). However, it seems that the information is not reaching consumers adequately or that consumers do not believe in the enforcement of such regulations. As a result, consumers value fish labelled as chemical, antibiotic or contaminant-free or possessing other safety certifications[36,38,39,43-46].

Despite the established perception that the aquaculture industry may use chemicals in their processes, consumers perceive farmed fish as being under stricter control than wild fish [22,26,47]. In addition, wild fish may be perceived as being more affected by pollution [48-50]. This leads to the belief that farmed fish is generally perceived as safe and trustworthy in the overall balance, at least at the same level as wild fish on this matter [28,34,36,37,47,51-59]. Runge et al. [60] observed that surveyed Wisconsin (USA) consumers had higher trust in fish farmers to keep fish safe if they trusted government agencies to do so. Attention to environmental news and conservative ideology were positively related to trust in fish farmers, and a preference for GMO-free/organic foods was negatively related to trust. Still, some reports contradict the general trend. In these, wild fish was perceived as safer or cleaner [23,61-65]. Some are from developing countries, which often face issues regarding seafood safety, albeit the improvement in hygiene conditions in the food industry over the years [66]. However, the concept of the superiority of wild fish in terms of safety might still linger in the consumer's perception even after regulations regarding food hygiene are enforced. Additionally, in these reports, consumers may attach greater importance to the risk of chemical contamination in their perception of safety.

3.3. Perceived quality

Experienced consumers claim to be able to evaluate the quality of the fresh fish through its appearance, based on different intrinsic cues of fresh fish: it should be bright-eyed, with pink gills, a fresh appearance and a pleasant smell [67]. After cooking, these would then be reflected in a high-quality product with a pleasant taste and texture. Less experienced consumers regarding seafood products might struggle in this assessment, often relying on extrinsic aspects, including the price, salesman credibility, and labels to assess the quality of a fish product [67]. Perceived quality is often claimed as one of the most, if not the most, important factors when purchasing fish [17,68-79]. Several studies have reported that the majority of consumers perceive farmed fish as having a lower quality than wild, associating farmed fish with a worse taste, texture or freshness [19,22,23,25-31,36,37,47-49,51,53,54,58,59,62,71,76,80-92]. Indeed, wild fish remains a standard for quality in fish products. One work in Myanmar reported that farmed fish sensory quality was harder to discern and that consumers relied on wild fish for more valued and traditional recipes, such as curries, while they used farmed fish mostly for frying [81]. It is worth noting that fish consumption frequency may play a role in this matter. Reig et al. [26] stated that for occasional (less than twice a week) fish consumers, a worse taste was one of the negative aspects of aquaculture. Still, for regular (over twice a week) consumers, the acceptable taste was one of the positive aspects. This is in agreement with the work of Verbeke et al. [53], who reported that light fish consumers (less than once a week) consider wild fish to have a better taste, while heavy fish consumers (over once a week) believe wild fish is fresher, but are unsure if this reflects on quality. Allegro et al. [51] also reported a higher percentage of consumers disagreeing with farmed fish being at the same quality level as wild, particularly among low-involvement consumers (those who consume fish once a month or less) compared to high-involvement consumers (more than once a week). Pupavac et al. [78] also showed that low fish intake consumers (up to once a week) believed farmed fish to be fattier and of poorer quality than wild fish. In contrast, high-intake consumers (over once a week) were uncertain, despite both groups generally preferring wild fish. Restaurant cooks and chefs also prefer wild fish over farmed [87], but they are willing to serve farmed fish if its sensory quality meets the standards expected by consumers [70].

Positive perceptions of aquaculture products' quality have also been reported [52,73,93-95], with consumers perceiving these products as having acceptable or high quality, coupled with a favourable price-to-quality ratio. The perception of aquaculture quality does not appear to be related to any fish species, although not many studies have assessed that issue in depth. Overall, this could suggest that consumers do not associate a worse image of farmed fish with a particular species, but if a species is farmed, its image may be affected. However, one work studied perception related to roho labeo (*Labeo rohita*), catla (*Catla catla*), pangasius (*Pangasius pangasius*) and Asian stinging catfish (*Heteropneustes fossilis*), showing a negative perception associated with farmed pangasius [96]; another study showed that for traditional Myanmar dishes, while consumers generally prefer wild fish, young consumers already tolerate some farmed species such as mrigal carp (*Cirrhinus cirrhosis*) or roho labeo, but not Nile tilapia (*Oreochromis niloticus*) [81]. Similarly, Menozzi et al. [97] showed that a stronger effect of the preference for wild specimens was seen for European seabass (*Dicentrarchus labrax*), compared to cod (*Gadus mouhua*), herring (*Clupea harengus*), pangasius, Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and gilthead seabream (*Sparus aurata*).

Further studies on the main determinants of quality aspects for consumers are needed for the aquaculture industry to effectively address those interests. These determinants may encompass extrinsic attributes perceived by consumers as requiring improvement in farmed fish. For example, Ribeiro et al. [84] observed that Portuguese consumers desired improved farmed fish's flavour, texture, and fat content.

Nevertheless, sensory attributes do not consistently justify the perceived lower quality of farmed fish. For example, in a blind test analysing smoked salmon (wild-caught, farmed or organically farmed), the overall liking of farmed fish samples surpassed that of wild fish [98]. Similarly, the overall liking of farmed fish samples in a blind test by Claret et al. [48] was higher than that of the wild samples. Drake et al. [99] compared saltwater farmed, freshwater farmed, and wild Southern flounder (*Paralichthys lethostigma*), observing a preference among the untrained panel for saltwater farmed over wild and freshwater farmed. In a similar fashion, Lawley et al. [100] assessed overall liking of wild, imported, sea-cage farmed, earth pond farmed and tank farmed barramundi (*Lates calcarifer*) and observed that sea cage farmed and earth pond farmed were valued above wild-caught fish. Rickertsen et al. [64] found French consumers to score farmed cod above wild regarding their sensory properties. Kole et al. [91] also showed that when Dutch consumers tried unlabelled cod fillets in a real-life setting, farmed fish was better appreciated, being less fatty and firmer. Farmed smoked horse mackerel (*Trachurus mediterraneus*) was also better evaluated than its wild counterpart by a panel, according to Koral et al. [101]. In the work of Kralik et al. [86], 63 fish consumers found no differences between wild-caught and aquaponic-raised yellow perch (*Perca flavescens*) concerning sensory properties. For wild and farmed brown trout (*Salmo trutta*), Antão-Geraldes et al. [102,103] reported no differences in their sensory assessment. In contrast, Pohar [104] found that 52% of the panel preferred farmed trout, favouring their texture, even though wild specimens displayed a better aroma and appearance. However, Guy and Nottingham [105] found that the flavour of wild mulloway (*Argyrosomus japonicus*) was preferred to that of farmed fish. Sylvia et al. [106] found that consumers preferred wild chinook salmon (*Oncorhynchus tshawytscha*) to farmed salmon in a blind test, citing its more delicate and fresh flavour.

This suggests that many conditions during farming, including fish species, rearing environment, water characteristics, feed, and other factors, play a significant role in the sensory properties of fish products, potentially outweighing the impact of the production method (farmed or wild) *per se*.

3.4. Price and availability

The decline in wild fish stocks, caused, among other factors, by overfishing, resulted in lower available wild fish products and a corresponding increase in their prices. This is particularly relevant as "price" is one of the foremost dimensions influencing food choice [107]. Aquaculture has emerged as an alternative, yielding high production volumes at lower prices.

Additionally, considering aquaculture offers tighter control on production conditions and leverage technological advancements (such as the development of recirculating aquaculture systems and other land-based systems), it can help mitigate product seasonality and facilitate the cultivation of exotic fish species away from their natural geographical habitats.

These concepts appear clear to consumers, and generally, farmed fish are perceived as cheaper than their wild counterparts [22,23,28-31,34,47,49,50,52,53,80,81,93,94,108,109]. This idea extends from the public to wholesalers, experts and the scientific community [21,26].

Price considerations are particularly important for consumers who value lower priced products, or value for money [43,51,63,69,71-74,82,85,90,93,110-120]. The perception of fish as a high-priced product represents a barrier for those who do not consume it frequently [51,71,75,121]. Price was also seen as a concern regarding the consumption of organic aquaculture or aquaponics products [122,123].

While Regnier and Bayramoglu [124] and Rodríguez et al. [125] observed that wild European seabass and gilthead seabream are more expensive than their farmed counterparts, two reports claim farmed fish as having a higher price in Germany [126,127]. The authors believe this situation in Germany may be caused by domestic stock shortages and the increasing demand for fish in that country, leading to the importation of greater amounts of farmed fish, with added transportation costs, thus being associated with production and supply issues. In addition, Regnier and Bayramoglu [124] suggest consumers may be more sensitive to production processes when it comes to higher-value species. Contrary to this, Genschick et al. [128] observed that in Zambia, the poorest of the poor households, unlike the better-off households, are less likely to supplement their diets with fish products from aquaculture and more inclined to consume products from capture fisheries, due to the higher availability and consequently lower price of small fish from captures in that country. However, the price matter is a double-edged sword. While some consumer segments value the lower prices of farmed fish, others see price as a quality cue and infer a lower quality of farmed fish from this [23,37,69,91,110].

Farmed fish are perceived as more easily available than wild, valued by consumers who appreciate year-round availability or species diversification [22,26,28-31,36,47,52,53,55,81,88,94,96,129]. Alam and Alfnes [96] observed that product availability was the 4th most important attribute for farmed fish purchasing, while being the least important for wild, reinforcing that availability is valued by consumers of farmed fish. Similarly, Mitra et al. [23] showed that consumers who value exotic fish species are more likely to prefer farmed fish, relating aquaculture to a higher species diversity. Currently, 56% (in weight) of the aquatic animal food production available for human consumption is provided by aquaculture [2]. This is highly dependent on geographical region and species, as some are still almost exclusively obtained from catches. As an example, in the EU, catches are responsible for almost all groundfish and flatfish for apparent consumption, but 94% of salmonids for consumption are farmed [9]. Thus, in the EU, aquaculture only represents approximately 30% of the *per capita* apparent consumption of fish products [9].

3.5. Perceived health and nutritional properties of fish

Different studies in Western countries have shown that health and nutrition concerns are important individual food choice criteria [107]. In fact, an increasing number of consumers believe that food directly contributes to their health, and that eating healthy products may help prevent nutrition-related diseases and enhance both physical and mental well-being. Fish and seafood were recognized by consumers from five countries (Brazil, France, Portugal, Spain and Uruguay) as essential components of a healthy diet, alongside fruits, vegetables, grains and cereals [130]. Indeed, fish is considered as one of the healthiest protein sources for consumption. In addition to protein, fish is known to be rich in polyunsaturated fatty acids (PUFAs), particularly the long-chain fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), along with a number of bioactive peptides coded within the fish proteins [131,132]. The benefits associated with fish consumption include prevention of cardiovascular diseases, ulcerative colitis and hyperlipidaemia, neuroprotective, antioxidant and anti-inflammatory effects, hepatoprotective activity and gut microbiota modulation [3,131].

Therefore, consumers generally perceive fish as a healthy and nutritious food, which is one of the motivations behind purchasing this product [18,43,77-79,121,133,134]. Indeed, Altintzoglou et al. [135] found a positive correlation between involvement in health issues and fish consumption, with this effect being notorious for both farmed and wild fish. However, once again, several reports found a difference between the perceived nutritional and health aspects of wild and farmed fish. Consumers see wild fish as healthier and more nutritious [19,23,28-31,37,48,51,53,58,64,65,84,86,88,92-94]. Concerns about the presence of chemical residues in farmed fish products may significantly contribute to this perception (section 3.2.). Moreover, in the work of Claret et al. [28], consumers perceived wild fish as being less fatty than farmed fish. This belief in the superiority of wild fish may stem from the belief that farmed fish is higher in fat content and lower in protein and polyunsaturated fatty acids.

A fish's diet plays a significant role in the nutritional profile of its fillet, impacting factors such as lipid and protein content, as well as fatty acid and amino acid profiles. As a result, there is substantial variability observed between feeding regimes and production methods. Mairesse et al. [136] also found that the nutritional quality of Eurasian perch (*Perca fluviatilis*) was influenced by geographical origin and season; wild perch from Lake Geneva had a similar fat content as intensively farmed, and these values were above those observed in wild fish from the Rhine River or non-intensive farms. In that work, the fatty acid profile exhibited significant variation depending on the farming system (intensive, semi-extensive and extensive) as well as the origin (Lake Geneva or Rhine River) of wild species. Consequently, a clear demarcation between differences purely attributed to farmed and wild fish was not delineated. Moreover, a meta-analysis by Sales [137] from 39 studies showed that differences in contents of EPA + DHA, total n-3 fatty acids, and the n-3:n-6 ratio in seawater fish due only to production method (wild vs. farmed) were mostly absent. However, the author claims farmed freshwater fish displayed lower total n-3 fatty acids content compared to their wild counterparts. Several subsequent studies have been published also claiming comparable fatty acids profiles and nutritional compositions between farmed and wild fish for similar sized specimens [138-140]. Moreover, some studies report that although some farmed species had lower percentages of fillet PUFAs, they also had higher fat concentrations, resulting in higher values of PUFAS, such as EPA or DHA, per portion [141,142]. A study on the common sole (*Solea solea*) showed that the sum of EPA+DHA on farmed fish was twice the value for wild fish [143]. Other studies reported better fatty acids profiles in wild specimens [144-146]. Overall, this suggests a high variability depending on species, feeding regime of the farmed fish, geographical origin, fish size, maturation stage, seasonality and genetic predisposition, which indicates farmed fish can be at the same level as wild in nutritional value and health-promoting effects, given adequate farming conditions.

In any case, some reports already indicate a more positive perception among consumers regarding the health impacts of farmed fish, considering them to be healthy and placing them at the same level as wild fish [34,50,52,55,56,108,109]. Low objective and subjective knowledge regarding farming practices and their subsequent impact on the health and nutritional characteristics of farmed fish have been reported [147], potentially explaining these discrepancies.

Considering that food health benefits are credence attributes, not directly and immediately experienced by consumers, the emphasis on health as a criterion for food choice hinges on effective communication and how consumers perceive various cues [148]. Therefore, when it comes to labelling and advertising, it is crucial to highlight the health advantages of fish products. To ensure effective communication, messages regarding health effects or specific compounds should be presented straightforwardly, considering consumers' generally limited knowledge about this matter [149,150].

3.6. Naturalness of fish products

Generally, consumers prefer foods perceived as natural because they associate them with healthiness, tastiness, freshness, and eco-friendliness [151]. New ingredients or new food technologies may decrease the naturalness perceived by consumers, particularly if consumers are not familiar with such ingredients/technologies [152]. Consumer acceptance of new food products or technologies depends on the balance between perceived risks and benefits [153], often leading to contradicting feelings: while advancements may be perceived as a way of increasing well-being, they can also evoke apprehension about unforeseen risks beyond individual control [154].

Aquaculture is usually perceived as an artificial production system, with farmed fish often regarded as unnatural products as opposed to the more natural wild fish. This perception can elicit feelings of disgust among consumers [26-28,47,50,51,53,88,155].

The study developed by Evans et al. [156] that evaluated consumers' ratings of the natural and unnatural attributes of foods, showed that: the concept of contagion accounts for the perceived naturalness of a product (adding something to a 'natural' base product reduces its perceived naturalness); chemical alterations to food products tend to have a more significant effect on reducing their perceived naturalness compared to physical processing; both the process through which food is produced and its composition affect a product's naturalness; products are perceived as more natural when they contain ingredients or components that are similar in nature; the more processing, the greater the reduction in perceived 'naturalness'; the use of E-numbers in label additives was perceived as less natural compared to using their chemical or common names; novelty does not necessarily imply a reduction in naturalness. Transposing this information to aquaculture, the association between farmed fish and the use of chemicals leads to a negative perception of this product. Additionally, including natural and marine products, such as algae, into aquafeeds has the potential to improve the perception of the naturalness of farmed fish. Moreover, Yip et al. [88] observed that fish products obtained from integrated multi-trophic aquaculture were preferred to conventional farming due to being perceived as more natural, which highlights the potential of new sustainable farming methods to counteract the negative image of aquaculture.

The farming processes and associated technology play a crucial role in shaping perceptions of farmed fish. Recirculating Aquaculture Systems (RAS) are tank-based systems that minimise water consumption through recirculation and treating water through biofiltration. They offer tight control over farming conditions, resulting in increased biosecurity and often leading to higher yields; they can also prevent the dissemination of diseases between farmed and non-farmed species [157,158]. The tight control on water quality parameters can also result in less stress for the animals. Thus, they are already in place for the production of some species, including Atlantic salmon, rainbow trout, barramundi, and European seabass, among others [159]. Still, RAS operations do come with some challenges associated with their high initial implementation costs and energy needs [157,158]. However, as technology improves, the benefit from RAS high productivity, low water consumption and improved animal health and welfare makes them attractive options. From the consumer's perspective, they are more industrialized, and consumers tend to draw a parallel from intensive livestock farming, thus often opposing this system [20,69,160,161]. Communicating the advantages of RAS could be an option to minimize aversion to this farming method, highlighting the water-saving and pathogen-protection properties of this system.

3.7. Ethical aspects: environmental, sustainability and welfare issues

The inclusion of ethical considerations into marketing strategies has become more and more common due to the growing interest in products and practices that are ethical and environmentally friendly [162]. Indeed, ethical convictions have been shown to affect both the perceived quality and perceived customer value of fish products [57]. Furthermore, environmental concerns are one of the reasons for minimizing or abstaining from consumption of fish and seafood [19,75,163,164]. Several studies reported that environmental considerations are important drivers of fish purchasing decisions [70,163,165-167].

Fishing is generally perceived as being eco-friendlier than aquaculture, leading to the favouring of wild fish [27,29-31,51,83,88,96,147,168]. Consumers are worried about the environmental impacts of fish farming, including the usage of hormones and other drugs, water consumption, pollution, disruption of wild stocks, farming effluents and habitat destruction [35,50,95,109,114,147,165,168-170]. Olsen et al. [171] showed that nearly 40% of Norwegian participants in a survey expressed scepticism regarding the sustainability of salmon production, and almost 50% did not believe in the environmental sustainability of the aquaculture industry as a whole.

Consumers tend to draw a parallelism between livestock farming or agriculture and fish farming, largely due to the low knowledge regarding the environmental impacts of aquaculture [27,50,88,147].

Dissemination of accurate information regarding aquaculture and its practices has the potential to improve the perception of fish farming. However, breaking this bias could be challenging: the work of Longstaff [172] showed that despite receiving explanations about the use of genetically engineered salmon feeds, concerns regarding the environmental impacts of salmon farming persisted. However, there is already a comprehension that aquaculture plays a significant role in minimising the impacts of overfishing caused by traditional fisheries [37,50,96,109,147,155,168,173]. In fact, some works already show that aquaculture was perceived as more environmentally friendly than traditional fisheries [59,64,89,93,174].

In addition, social norms can play a significant role in purchasing certified aquaculture products. Yi [175] observed that for consumers with high environmental awareness, attitude (the degree to which one assesses a certain behaviour as favourable or unfavourable) was more relevant towards behavioural intention than social norms. However, for low-awareness consumers, social norms were more significant.

3.7.1. Perceptions of emerging farming methods

Emerging farming methods, that focus on sustainability, can be perceived as more environmentally friendly [20,111]. For example, organic fish farming has become increasingly relevant. In the US, there are currently no federal standards for organic certification (it relies on private certification) [176], but in the European Union, regulation (EU)2018/848 on organic production and labelling of organic products is already in force. According to this, organic production should contribute to the protection of the environment and climate; promote biodiversity; rely on high animal welfare standards, meet the species-specific needs of animals; encourage short distribution channels and local production; respect Nature's systems and cycles; use responsibly energy and natural resources [177]. As such, it comes as an alternative to traditional farming, addressing many of the consumers' concerns: minimizing the usage of 'chemicals', increasing animal welfare, and protecting the environment, among others. Feucht and Zander [20] found, in a focus group with German consumers, that organic fish farming was perceived as the ideal practice, and indistinguishable from sustainable aquaculture. Indeed, associations between both sustainability and the environment with organic aquaculture were frequently reported [24,55,111,113,178]. Organically farmed fish was also seen as healthier [24] and with a higher quality [33,68,70,98,179]. Thus, consumers are willing to pay an added value for fish products labelled as organic [24,35,55,112,178-182].

Another aquaculture practice that has grown in popularity is Integrated Multi-Trophic Aquaculture (IMTA). In short, uneaten feed and waste from one species are used as nutrients for another [183]. This way, fish could be farmed simultaneously with shellfish and/or seaweeds, increasing the aquaculture processes' sustainability and reducing their environmental cost [184]. The environmental and sustainability benefits are perceived positively by consumers and stakeholders [88,109,114,118,185]. Moreover, an issue that has been mentioned in several reports is the lack of awareness regarding IMTA [88,109,114,118,185]. While consumers are familiar with the "organic" concept, which is widely diffused due to its application to other sectors, including dairy, meat, vegetables, etc., IMTA is a rather specific aquaculture practice, and still quite unfamiliar to consumers. However, several stakeholders claim IMTA can contribute to the improvement of aquaculture perception if the information is adequately disseminated [185].

Aquaponics is a specific integrated multi-trophic system in which the nutrient-rich water from the tanks is used for plant growth [186]. Thus, it also promotes sustainability, considering the recirculation of water, nutrients and minerals [186]. The positive impact on the environment is recognised by some consumers [44,123]. Moreover, aquaponic products were reportedly perceived as healthier and with a higher quality [33,86], and some segments would be willing to pay a premium for them [33,187,188]. Finally, as was the case with IMTA, lack of information is still a barrier [44]. Kralik et al. [86] found that providing respondents with information regarding aquaponics increased the purchase intention to levels above wild fish.

3.7.2. Certification of production systems

Both Weitzman and Bailey [189] and Chikudza et al. [190] performed interviews with several stakeholders regarding the impact of eco-labels certifying production systems. While several believe presenting these certifications in labels can aid as a communication tool and lead to a valorisation of the products, others question their credibility, due to inadequate standards and consumer confusion due to the increasing number of eco-labels in the market. Indeed, several other reports show consumers to be unaware of eco-labels and certification systems, confusing them, or believing fake labels to be real [19,20,69,111,129,169,170,191]. Feucht and Zander [20] observed that consumers question the credibility of such labels, lack sufficient knowledge to appreciate their value, and find the process of seeking them out to be time-consuming. However, in the work conducted by Ariji [192], a hypothetical eco-label for bluefin tuna was well accepted by Japanese consumers.

Regarding farmed fish, they can attain organic certification, which can be conferred by either national or private institutions (for example, the Naturland logo in Germany, or the KRAV logo in Sweden) or indicated by the EU organic logo (certified by an authorised control agency or body). Alternatively, they can be certified by the Aquaculture Stewardship Council (ASC), which requires farmers to conform with performance-based standards. Third-party certification organizations verify the farm's compliance with such standards. After the independent assessment and certification of the farm as "responsible" on the environmental and social aspects, the farm may apply the ASC label on its products [193]. This label came as a response to the Marine Stewardship Council (MSC) certification scheme, referring only to wild fish [194].

Studies show consumers are more familiar with the MSC certification, and often confuse MSC and ASC [19,20,69,191]. The effects of the ASC label are varied. Some reports show that this is not significant for consumers, which may favour other certifications (such as organic) [19,110,181]. Others show a positive valuation for farmed fish certified as sustainable through the ASC logo [112,119,169,195,196]. Chen et al. [197] compared the MSC label for wild cod with a French biological label for farmed cod in a survey with French consumers and observed a higher premium for the biological certification than for MSC. Again, a higher familiarity with the biological/organic concept is thought to be responsible for this result. Interestingly, the same study showed that eco-certifications alone might not be enough to mitigate the effects of negative environmental information, suggesting a potential lack of credibility associated with these certifications.

3.7.3. Sustainable aquafeeds

Aquafeeds can represent between 40-60% of production costs [198] in the aquaculture industry. Uneaten or poorly digested feeds are also responsible for a large portion of waste from fish farms [199]. Furthermore, the inclusion of high levels of fishmeal (FM) and fish oil (FO) has been considered unsustainable in the long term, as they are still mostly obtained from the capture of pelagic fish, and the intensification of aquaculture has caused pressure on wild stocks due to the increasing demand for FM and FO [13]. Thus, attempts have been made to replace these ingredients, using other protein and lipid sources in aquafeeds, such as vegetable or insect meals and oils [200,201]. However, generally, that information is not available on fish labels. One study determined Singaporean consumers believe fish fed alternative aquafeeds should be labelled as such, despite supporting funding to develop such feeds [202]. Italian consumers also support further research on novel aquafeeds [203]. In general, consumers would likely be open to eating fish fed novel aquafeeds, such as those derived from insects, as long as the quality and safety of the final product remain unaltered [112,203-206]. Nevertheless, consumers generally have limited knowledge about aquafeeds, including their composition and associated problems [20,26,172,204,207]. Some common preconceptions include association between FM and FO with cannibalism [20]; concerns about the possibility of illness transmission due to FM and FO (similar to the case of bovine spongiform encephalopathy) [20]; unawareness of the possibility of using insects as feed ingredients [204,207]; belief that wild fish have a healthier diet [47,48,92]; and belief that aquafeeds are not natural [27]. Interestingly, Baldi et al. [206] reported that Italian respondents, over the age of 55, found plant-based aquafeeds to be the least natural, compared to FM and insect-based feeds.

Several reports found aquafeed composition insignificant for the economic valuation of farmed fish [55,79,85,112]. This is in line with an opinion stated by a fish farmer in the work of Popoff et al. [204], who indicated consumers were more interested in healthy, safe and sustainable fish products rather than being specifically concerned with fish feed. Moreover, Arru et al. [208] found that Italian consumers showed considerable price sensitivity, making it challenging to transfer the additional costs associated with using sustainable alternative feeds onto them. In contrast, Ferrer Llagostera et al. [83] and Hoque [165] found that consumers are willing to pay premiums for fish fed insect-meal-based feeds or other sustainable alternatives (with lower feed conversion ratio, FCR). These studies, nevertheless, posed a strong tone on the sustainability issue. Additionally, Baldi et al. [205] observed that environmental attitude was a predictor of acceptance of insect-based aquafeeds among young consumers. Similarly, in the work of Rosenau et al. [79], "fed with sustainable resources" emerged as the sole significant feed claim, with others such as being "fed without FM", "fed on plant-based proteins", and "fed with spirulina" showing no significance. Thus, this suggests what consumers value is an overall increase in sustainability. However, a study with older consumers in Italy contradicts this idea: the surveyed sample expresses a general lack of concern regarding the environment impact of fish farming. Instead, they worried more about the feeding practices employed in farmed fish production, possibly due to fears of the impact of feeds on the nutritional properties of fish [206].

3.7.4. Fish welfare

There is a growing concern regarding animal welfare, and this has become extremely significant for the animal production industry. Consumer avoidance of animal food products often steams from prioritising animal welfare [209,210]. The question of whether fish, unlike mammals, can experience pain and suffering similar to humans has sparked debates within the aquaculture sector. Studies focusing on fish have unveiled the presence of nociceptors, receptors responsible for detecting tissue damage or potential threats to tissues. Remarkably, these nociceptors exhibit properties akin to those found in other mammals and humans [211]. The definition of welfare itself has caused debate. The UK Farm Animal Welfare Council established five essential “freedoms” in 1979 for animal welfare: freedom from hunger/thirst; freedom from discomfort; freedom from pain, injury and disease; freedom to express normal behaviour; and freedom from fear and distress [212]. These principles have been generally adopted as key indicators of the welfare of production animals. Specifically for farmed fish, Stien et al. [213] have defined five essential needs for these animals: adequate nutrition, appropriate water quality, good health, behavioural freedom and safety.

Some regulations are already in place to address fish welfare concerns. For example, in the EU, several countries have incorporated Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes into their regulations [214]. In countries such as the US or China, there are currently no specific regulations regarding fish welfare [215,216]. However, the aquaculture industry has accompanied the growing interest in this matter, and many aquaculture companies seek external certification to demonstrate their compliance with fish welfare guidelines.

Studies confirm the public’s interest in farmed fish welfare [20,35,96,167,169], and even that some consumers express a willingness to pay an added value for fish farmed according to specific welfare standards [32,35,181,210,217]. A study by Hoerterer et al. [21] showed that respondents with a scientific background were less inclined to agree with the statement, “Aquaculture is not good for fish welfare, but it is the only way to ensure seafood availability”. In contrast to these views, a study conducted in Finland challenges these ideas, claiming that there is no immediate consumer pressure to improve farmed fish welfare. This lack of urgency is attributed to consumers’ believing that current welfare standards are already acceptable and that fish (salmon) possesses lower mental abilities compared to superior vertebrates [218]. On a similar note, Allegro et al. [51] observed that 55.5% of the surveyed respondents partially agree, while 20.1% fully disagree, that fish farming ensures animal welfare. Additionally, when comparing the welfare concerns of farmed fish with those of farmed pigs, Dutch consumers displayed greater apprehension about the welfare of pigs, as fish were perceived as being less likely to experience emotions [219].

Moreover, the idea that wild fish have better welfare than farmed fish is widely accepted by consumers all over the world [21,32,52,53,96,111,160,169,174,210], despite, Hartmann and Siegrist [220] observing no differences in how morally justifiable respondents perceived farmed fish and wild fish production. In a survey of Spanish consumers, the average agreement level with the statement: "I have no ethical concerns eating farmed fish" was 4.9 on a scale of 1 to 7 [221]. Conversely, the agreement level with statements such as "fish farming violates animal rights" and "slaughtering of farmed fish causes unnecessary suffering for the fish" averaged 3.1 in the same study. Kalshoven and Meijboom [129] observed, in interviews with stakeholders, that welfare was perceived as being more associated with the slaughter moment both for wild and farmed fish. Thus, farmed fish were thought to have better welfare, because they were thought to be stunned before slaughter.

Most consumers lack awareness of fish welfare issues. While they generally agree that animals should not undergo unnecessary suffering, consumers are unfamiliar with regulations or common aquaculture practices concerning animal welfare [34,63]. Thus, in addition to enhancing regulations regarding fish welfare, there is a pressing need to further dissemination of aquaculture practices that minimize harm to fish welfare and improve the perception of aquaculture products. Moreover, the work of Olesen et al. [24] showed that 53% of surveyed Norwegian consumers partly or totally agreed that organic production better promoted fish welfare compared to conventional methods. This suggests that investing in organic production could potentially help shift this negative perception.

3.8. The role of information

One clear observation from many of the analysed studies is the great public unfamiliarity with aquaculture. Consumers are generally unaware of technological advancements [19,20,69,88,109,114,170,178,222], aquaculture standards [27,34,63,83,122,169], sustainability and environmental impact [19,27,29,34,50,88,147,164,168,170,223], feeding practices [52,83,172,204,207], and even the origin of the fish products they buy [21,28-30,51,52,89,90,100,135,173,223-226]. In many cases, consumers are not even aware of the existence of farmed fish [49,53,89].

Both objective (the actual level of knowledge an individual has about a product) and subjective knowledge (perceived level of knowledge) affect consumer's perception of aquaculture products, their purchasing intentions, and their behaviour. It is worth noting that individuals are aware of their own lack of information: subjective knowledge regarding aquaculture tends to range from low to medium levels [17,21,26,27,47,74,78,83,92,100,111,114,134,164,165,170,171,173,178,217]. In this sense, Olsen et al. [171] showed that 40% of Norwegian respondents found it challenging to obtain information about the salmon aquaculture industry. Often, individuals draw inferences based on their existing knowledge about agriculture [28,52,53,69], suggesting that perceptions of aquaculture are often stemmed from emotions than from awareness and factual knowledge. A similar situation occurred among responsible personnel at single-unit supermarkets and restaurants, who mostly base their farmed fish ratings on perceptions rather than actual knowledge [87]. Hoque and Alam [227] observed a negative correlation between positive knowledge discrepancy (having a higher subjective knowledge than objective knowledge) and attitudes towards farmed fish. Conversely, Menozzi et al. [92] observed that subjective knowledge had a negative impact on attitudes toward farmed fish while objective knowledge had a positive effect. Thus, overestimating knowledge leads to more negative attitudes towards farmed fish, supporting the idea that negative perceptions of farmed fish are often more based on emotions than facts.

Increased knowledge about fish seems to be associated with higher fish consumption rates [228,229]. Furthermore, some reports suggest that a greater objective knowledge might relate to a better perception of aquaculture [47,53,92]. In Bangladesh, subjective knowledge was also positively correlated with the intention to purchase farmed fish, while objective knowledge did not show significant correlation [227]. In contrast, Jonell et al. [170] showed that both objective and subjective knowledge were weak predictors of eco-labelled seafood purchases. Similarly, and despite the impacts of objective and subjective knowledge on attitudes towards farmed fish, neither was correlated, as described by Menozzi et al. [92].

These findings collectively suggest that while knowledge about fish and aquaculture can impact consumption habits and perceptions, the relationship is complex and may vary depending on cultural contexts, labelling practices, among other factors.

Presenting information related to the aquaculture process to consumers prior to their perception assessment can have a significant impact on their perception of aquaculture and their intention to purchase farmed fish [34,108,192]. Similarly, informing respondents that farmed salmon has a higher content of omega-3 fatty acids improved their perception of farmed salmon in comparison to wild [230]. Kralik et al. [86] observed that providing information about aquaculture and aquaponics boosted the purchase intention of Ohio consumers for such products, surpassing even the levels observed for wild fish, particularly in the case of aquaponics. In addition, providing information associating fish welfare with organic production increased the willingness to pay for organic products, while information associating organic production with environmental concerns did not have the same effect. This may be because consumers are already familiar with the association between organic production and environmental benefits. [181]. Baldi et al. [206] and Bazoche and Poret [207] also showed that providing respondents with information about aquafeeds and their environmental impact increased acceptance of insect-based aquafeeds. In the study by Bronnmann and Asche [195], after providing background information on salmon production methods, sustainability challenges in fisheries and aquaculture, and the certification criteria of the MSC and ASC, the preference for farmed fish was found to be even lower than before participants being informed. However, information improved perceptions of certified salmon, with the ASC having a higher value than the MSC. This suggests that consumers are more willing to consume and pay for farmed fish once they are informed about sustainable farming methods and certification. However, this is not consistent with results achieved for farmed and labelled turbot, where information about ASC certification was not enough to increase its acceptance [19]. Similarly, Kole et al. [91] showed that providing positive information about fish farming did not increase the overall liking of farmed cod products, but rather decreased it (compared to equal products labelled as farmed without additional information). One possible explanation for these results is that the information was not conveyed effectively to the participants in these two studies. In any case, the literature seems to highlight of importance of disseminating information to consumers as a way to increase knowledge and thus improving aquaculture acceptance.

It is worth noting that some studies show that simply labelling fish as farmed, without providing additional information on the production system characteristics, decreases their overall liking compared to wild fish. Interestingly, when blind tasting is conducted, with no information provided on the production system, there are non-perceived differences between samples' overall liking [48,64,91].

Additionally, it seems that disseminating correct information is more important than the specific format in which that information is displayed [108,172]. Uchida et al. [230] tested different types of information regarding salmon farming and found that revealing the source of information (whether from universities, national agencies or industry) was less significant than the content of the information itself. However, the work of Altintzoglou et al. [56] showed that when respondents already have a positive perception of aquaculture, exposure to additional balanced information regarding safety, healthiness, and sustainability of aquaculture does not further enhance nor worsen that positive image.

Hansen and Onozaka [231] studied the spillover effects of negative publicity (associated with disease outbreaks on fish) on the valuation of fish products from unaffected countries and species. The authors found adverse spillover effects on the same species from unaffected countries and other species farmed in the affected country. Moreover, brand association with an upscale retailer was not able to repair the damage. In contrast, Chen et al. [197] showed that displaying negative environmental information on a certain farmed species improved the perception of other farmed species. As such, the spillover effects in the aquaculture context may vary and merit further investigation.

Mass media are a significant source of information about aquaculture, along with word of mouth [47,71,77,78,95,223], often more important than official channels, which may still pose an issue regarding the dissemination of correct information. Indeed, a study in Idaho and Washington, USA, found that approximately 50% of respondents recalled hearing or reading about aquaculture in the mass media and recalled particularly negative stories [147]. In this sense, one report analysed newspaper headlines regarding aquaculture [232]. It revealed that while headlines overall tend to be more positive than negative, developed countries had proportionally more negative headlines than developing nations. In particular, off-shore aquaculture is associated with negative opinions. The report also indicates that environmental disturbances unrelated to aquaculture, such as oil spills, harm aquaculture perception. However, the authors note a shift in this tendency, with positive headlines becoming more frequent with time. It is possible that, if this tendency remains, public perception of farmed fish will become better and better. Another study explored how health risks and benefits are communicated in newsprint articles about chemical contamination in farmed salmon [233]. The vast majority of statements were classified as qualitative, and the authors suggest that readers could not establish specific interpretations. In addition, journalists' rhetorical practices transmitted contradictory interpretations of the same data, resulting in inconclusive messages about the risks and benefits of farmed fish. Considering the relevance of social networks for information dissemination, Baraibar-Diez et al. [234] analysed aquaculture communication through Twitter. They suggested that aquaculture producers and associations should use this network more often to appeal to consumers and that while they focus on product promotion, they could better communicate the benefits of their activity. Moreover, negative words are often used to convey positive messages, which may hinder the effectiveness. Finally, the authors suggest homogenization of hashtags and categories to facilitate user search. Overall, this highlights the significance of social and mass media as a way to spread information regarding aquaculture, but caution is advised in the writing of the messages, to ensure the message gets through correctly.

Indeed, mass media and social networks may have a stronger impact than product labels, which consumers may confuse or misinterpretate [19,20,69,111,169,170,191]. Feucht and Zander [20] reported a lack of credibility of some labels, and consumers found that looking for information on labels was "time-consuming". In this sense, Bogliacino et al. [235] suggest that labels should be simplified (minimizing other claims that may distract the information from mandatory claims), clarified and verified by independent audits.

3.9. Importance of the country of origin

Consumer ethnocentrism has been well described in the literature and leads to favouring local, domestic, or national products over foreign ones. Ethnocentric consumers may believe purchasing foreign products is unpatriotic, harms the domestic economy and causes losses in domestic jobs. Domestic products are associated with a sense of pride and are perceived as being superior to foreign products. This is also the case for fish products, where the country of origin (COO) plays a very relevant role at the purchasing moment [43,71,72,79,90,100,110-112,115-117,164,179,223,236]. Worldwide, a tendency to prefer local or national fish was observed [17,20,35-37,43,44,55,64,69,71,79,90,110-112,114,116-119,161,167,169,179,181,192,236-240], particularly as local/national fish tends to be perceived as fresher. Thus, consumers may associate a shorter geographic distance travelled by the product with a higher freshness and quality [35]. Indeed, Hinkes and Schulze-Ehlers [169] found that German consumers perceive fish from Germany to be superior in product quality, food safety, social, and environmental standards fish from than Bangladesh and Vietnam. In addition, Garlock et al. [239] suggested that in USA, there is a higher probability of Gulf of Mexico farmed fish substituting imports than domestic wild-caught fish, given that American wholesale buyers prefer domestically sourced fish over imported, but they do not necessarily prefer wild-caught fish over farmed fish.

However, two reports contradict this tendency. Quagrainie et al. [241] observed that Indiana (USA) consumers who buy wild-caught fish are not interested in Indiana-farmed fish, as they prioritise production method over origin. Nevertheless, those already purchasing farmed fish would prefer local fish. Moreover, consumers may lose interest in Indiana aquaculture products as premiums increase. Similarly, Witter et al. [75] found that around 40% of Canadian respondents would not be willing to pay a premium for the "local origin" attribute, with only 4% considering it the most important factor for purchasing. Nguyen et al. [59] found the difference in perception between imported and domestic fish among US restaurant consumers was ambiguous.

Abaidoo et al. [240] showed that when consumers were exposed to news regarding seafood fraudulent labelling (imported fish labelled as having a U.S. origin), a spillover effect was observed, with a lower preference for US-labelled fish. Interestingly, authors found that this effect was not significant when the origin was labelled as from the Great Lakes.

3.11. Socio-demographic characteristics influencing consumer preferences

Several works show an association between older age and a preference for wild over farmed fish [22,38,47,52,53,59,64,72,80,90,109,122,227,242-244]. However, in the study of Ribeiro et al. [84] older consumers were more receptive to aquaculture products. Some works found age to be insignificant [48,62,224]. Hoerterer et al. [21] found older consumers to believe aquaculture to be more sustainable, but no correlation was found between preferring wild to farmed and age. In addition, high fish consumption has been associated with a preference for wild-caught products [21,38,52,53,58,72,88,121,134,243], despite studies by Krešić et al. [47] and Stancu et al. [224], who found the opposite. This overall suggests an association between wild fish and traditionalism: older individuals used to consume fish frequently may prefer the more "traditional" wild-caught fish.

While the roots of aquaculture trace back to 2070 BC in China, its development in Europe and the USA, became more prominent in the late 20th century [245]. As such, neophobia regarding farmed fish may still significantly influence the preference for wild fish, particularly in these regions, although not exclusively. For example, in countries like Turkey and Bangladesh, an association between higher age and preference for wild fish has also been found [38,80,242]).

Aquaculture is also often associated with convenience, leading to farmed products being preferred by young, working or student consumers (probably with a busier lifestyle) who value frozen and processed or ready-to-eat food products [23,47,71,80,243,246]. Nevertheless, Hoque et al. [242] observed that profession was not a significant predictor of farmed fish consumption.

Regarding education levels, higher education degrees were associated with a more positive perception of farmed fish [21,22,51,109,224,243]. However, Guney [80], Yip et al. [88] and Hoque and Alam [227] encountered the opposite, while other reports [23,47,48,53,62,242,244] show that education level is not a significant predictor.

Income appears to be a more divisive factor in seafood preference, with some reports suggesting that individuals with higher incomes tend to prefer wild fish, while low-income consumers favour farmed fish [38,53,62,71,88,242]. Conversely, there are contradictory findings [29-31,47,109,243], and some reports considering income to be insignificant [22,38,59]. Both behaviours can be justified. Wild fish is generally perceived as more expensive and thus preferred by those with a higher income, while low-budget consumers tend to resort to farmed fish. In this sense, Guney [80] showed that housewives, students, and unemployed are more likely to consume farmed fish, despite finding inconsistent results for income. On the other hand, higher incomes may be associated with higher education levels, leading to better acceptance of aquaculture products.

Consumers who live or were brought up in a coastal region also seem to prefer wild fish [51,226,244,247]. This can happen because in such regions, capture fishing is a traditional and significant economic activity, and aquaculture might be seen as conflicting with this.

The presence of children in the household was also associated with a preference for farmed fish [22,51,59,155,242], despite some works claiming this to be insignificant [47,62]. Additionally, Allegro et al. [51], Masi et al. [71] and Cantillo et al. [155] found that larger households (with 4 or more members) favoured farmed fish. This may be due to the perceived higher price of wild fish. In contrast, Mitra et al. [23] found that the probability of preferring wild fish increased with the number of family members. The authors correlated this finding with the fact that the probability of preferring wild fish also increases with the number of income-earning family members. Other authors [47,53,59,62,80,242,244] did not find household size to play a significant role in seafood choice.

Regarding gender, most studies found it to be insignificant [21,22,47,48,53,72,80,122,226,242,243], while others found male consumers to favour wild fish and female consumers to value farmed fish [27,28,52,62,244,247]. Other studies [29-31,59,84,155] found male consumers to be more supportive of aquaculture.

3.11.2. Impact of nationality on consumer's perception – country-comparative studies

Several reports compared consumer perceptions of farmed and wild fish from different countries, to explore whether this could significantly predict consumer behaviour. Results vary wildly. Most of these studies have focused on European countries. López-Mas et al. [22] found a more positive perception of wild fish compared to farmed fish in France than in Germany, Italy, the United Kingdom, and Spain. This aligns with the findings of Masi et al. [71], where French consumers displayed a strong emphasis on production method information. Menozzi et al. [97] also found that the preference for wild-caught fish was higher in France and Italy than in Germany, Spain, and the UK. Schlag and Ystgaard [50] observed that French participants perceived wild fish as very expensive, to the point of often being unobtainable, which can also reflect its perceived higher quality. In the work of Vanhonacker et al. [94], UK consumers had the most negative perception of farmed fish, compared to those from Czechia, Germany, Greece, Italy, Portugal, Romania and Sweden. Additionally, Schlag and Ystgaard [50] found UK consumers to have strong doubts about the safety of farmed fish. In general, Norwegian and German consumers had the most positive perception of farmed fish, possibly due to the strong dissemination of this product in those countries and the importance of the industry for their economy [50,56,94,168]. However, Stancu et al. [224] observed that the consumer segment with the lowest intentions to buy all new aquaculture products was mostly composed of German consumers. Nevertheless, this could be more related to their lower innovativeness rather than a German bias towards aquaculture. Altintzoglou et al. [135] observed that consumers from Belgium displayed a higher farmed-to-wild fish consumption ratio than Norway and Spain. Alexander et al. [185] found Cyprian stakeholders to be generally against aquaculture, citing its conflict with capture fisheries.

Consumers from all European countries are generally interested in increased sustainability in aquaculture [35,57,97,109,119]. Some differences were observed, but results are not always consistent. Nevertheless, three studies showed the importance of sustainability labels for German consumers [35,97,119]. van Osch et al. [118] found UK consumers to be willing to pay the highest premiums for sustainable fish, while Norwegian had the smallest values. López-Mas et al. [174] showed that French consumers were more neutral when classifying farmed fish as more sustainable and with increased environmental benefits than wild, compared to German, Italian, Polish and Spanish consumers. The same work showed that an ethical perception of aquaculture was less pronounced in Poland and France. Moreover, Pieniak et al. [223] observed differences in consumer objective knowledge depending on their origin, with southern European consumers having a higher knowledge compared to other European consumers. Similarly, Altintzoglou et al. [135] showed that Spanish consumers displayed a higher awareness of fish origin (farmed vs. wild) than Norwegian or Belgian consumers. Banovic et al. [57] observed that ethical beliefs had a stronger impact on perceived product quality for consumers from Spain, Italy and the UK compared to France and Germany.

Some reports found that geographical differences were rather minor [33,52,120,166,188,246]. Considering this and the strong variability observed when comparing multi-country reports, along with the globalization and migration tendencies worldwide, it is likely that other social and demographic parameters may be better predictors of the preferences for wild and farmed fish.

4. Overall consumer preference and willingness to pay

In general, there is a tendency for consumers to give preference towards wild-caught products [19,21,32,39,43,51,61-63,65,71,72,74-76,78,82,83,85,88,89,93,94,97,98,113,121,167,192,195,221,225,230,236-238,240,247-249]. This can be seen through answers to surveys and focus groups, where consumers claim to prefer wild-caught fish, or even when analysing the willingness to pay (WTP). Premiums for the wild fish attribute vary from \$1.1/kg to \$2.8/kg for wild roho labeo [39,61], \$2.76/kg, \$11.1/kg, \$12.84/kg or \$28.52/kg for Atlantic salmon [62,65,85,195], \$6.8/kg for turbot [19], \$7/kg to \$25.7/kg for gilthead seabream [43,83], \$4.3/kg for rainbow trout, yellow perch and walleye [238], \$24.1/kg for tuna [85], 77 yen/sushi piece for bluefin tuna[192], 1.68€/kg for cod [197], \$9.8/lb for arctic char and \$4.6/kg for moi [85]. Determining the WTP, particularly through the analysis of Discrete Choice Experiments (DCE), is important for understanding consumer behaviour, as these experiments closely mimic actual purchasing situations. Consequently, the generated results provide a better approximation of consumers' real buying behaviour [250].

Hoque et al. [39] found that production method was the most important attribute in purchasing fish. In contrast, according to a survey, Bronnmann and Hoffmann [19] reported that production method was the attribute to which consumers paid less attention. However, there was still a negative WTP for the "farmed" attribute. In Spain and Portugal, Fernández-Polanco et al. [43] and Pereira et al. [74] found that production method was the third most important attribute, after price and origin (in Spain) or external characteristics and species. Nevertheless, it still resulted in a positive WTP for the wild fish. The importance of this attribute was also observed when assessing the value of an edible invasive species in Portugal [249], which showed that consumers were willing to consume such species, particularly because it was wild-caught and they valued that attribute. Nevertheless, some reports contradict these ideas. In Bangladesh, a study by Alam and Alfnes [96] found that consumers are not willing to pay extra for wild fish. Kulesz et al. [222] claimed Swedish consumers evaluated farmed fish positively and they were not willing to pay an extra above \$1.7/kg for wild fish. This is in line with reports that claim consumers do not place significant importance on the production method [77,84,115,116,134,169,173,191,239,251,252]. From focus group studies, Saidi et al. [37] observed that interviewees from the North Mediterranean (Italy and Spain) did not value this attribute when purchasing. However, south Mediterranean interviewees in the same study preferred wild fish.

Two studies reported a neutral preference in the "wild vs. farmed" matter [52,244]. Some reports already show a segment of consumers having no preference between wild and farmed fish, despite a still significant fraction (over 30% of respondents) preferring wild fish [21,29-31,71,75,88,98,171,237,240]. One survey in Turkey reported a major fraction (72%) of respondents preferred farmed to wild fish [80], and another work in the landlocked state of Kentucky found a positive utility for farmed fish rather than wild [226], which are exceptions to the trends found. In any case, Cantillo et al. [155] showed that using negative statements in Likert scale instruments assessing the acceptability of aquaculture and its products may not be advisable, as they tend to bias results towards a more negative perception of aquaculture, with a higher variance and less reliability.

5. Possible strategies for improving consumer acceptance of farmed fish

Some recommendations can be made to improve consumer perception of farmed fish. Addressing consumer concerns regarding the presence of chemicals in farmed fish needs to be further tackled. One recommendation is to strengthen and enforce the current regulatory framework to ensure that products containing high concentrations of antibiotics and hormones are not available in the market. Communication of this regulatory framework to consumers needs to be more effective, as consumers are not fully aware it exists or if the industry complies with it. This correlates with a second point that is also extremely important: there is a pressing need to ensure the dissemination of correct information regarding aquaculture reaches consumers. Social networks present a valuable opportunity to facilitate the widespread dissemination of this information. Besides, promoting domestic aquaculture can capitalise on consumers' preference for local, regional, or national products, potentially increasing acceptance of farmed fish. Moreover, the aquaculture industry can benefit from market differentiation by offering higher-value products with perceived higher quality, considering that perceived product quality affects perceived customer value. Finally, adopting sustainable and eco-friendly practices, while prioritising animal welfare, is essential for the aquaculture industry. Products obtained through such practices should be clearly and objectively labelled to enhance their acceptance. This can be related to the prior suggestion, as eco-friendly fish products, such as organic or those obtained from IMTA, are also perceived as having a higher quality.

6. Conclusions

Overall, this literature review showed that the perception of wild-caught fish as a higher quality, more natural, and healthier product persists across several geographical backgrounds. Concerns regarding farmed fish, such as the belief in its high concentration of chemicals such as antibiotics or hormones, still hinder its acceptance. Generally, fish consumers display low objective and subjective knowledge regarding aquaculture, including its practices, standards, and products. In addition, both the production method and the country of origin significantly influence consumer purchasing decisions. Improving communication to consumers is crucial for increasing acceptance of farmed fish. In parallel, the industry should prioritise sustainable farming methods, which often address other consumer concerns. Finally, emphasising the local or national origin of farmed fish may also increase its acceptance among consumers.

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References

1. Hentrich S, Salomon M. Flexible management of fishing rights and a sustainable fisheries industry in Europe. *Mar Policy*. 2006;30(6):712-720. doi:10.1016/j.marpol.2005.11.003
2. FAO. *The state of world fisheries and aquaculture 2022. Towards blue transformation*. FAO; 2022. doi: 10.4060/cc0461en.
3. Chen J, Jayachandran M, Bai W, Xu B. A critical review on the health benefits of fish consumption and its bioactive constituents. *Food Chem*. 2022;369:130874. doi:10.1016/j.foodchem.2021.130874
4. Krittanawong C, Isath A, Hahn J, et al. Fish consumption and cardiovascular health: A systematic review. *Am J Med*. 2021;134(6):713-720. doi:10.1016/j.amjmed.2020.12.017
5. Almeida C, Karadzic V, Vaz S. The seafood market in Portugal: Driving forces and consequences. *Mar Policy*. 2015;61:87-94. doi:10.1016/j.marpol.2015.07.012
6. Shamshak GL, Anderson JL, Asche F, Garlock T, Love DC. US seafood consumption. *J World Aquac Soc*. 2019;50(4):715-727. doi:10.1111/jwas.12619
7. Fabinyi M, Liu N. The social context of the chinese food system: An ethnographic study of the Beijing seafood market. *Sustainability*. 2016;8(3):244. doi:10.3390/su8030244
8. Ferrari L, Panaite S-A, Bertazzo A, Visioli F. Animal- and plant-based protein sources: A scoping review of human health outcomes and environmental impact. *Nutrients*. 2022;14(23):5115. doi:10.3390/nu14235115
9. European Market Observatory for Fisheries and Aquaculture Products. *The EU Fish Market*. 2023. doi: 10.2771/38507.

10. Bush SR, Belton B, Little DC, Islam MS. Emerging trends in aquaculture value chain research. *Aquaculture*. 2019;498:428-434. doi:10.1016/j.aquaculture.2018.08.077
11. Little DC, Newton RW, Beveridge MCM. Aquaculture: a rapidly growing and significant source of sustainable food? Status, transitions and potential. *Proc Nutr Soc*. 2016;75(3):274-286. doi:10.1017/S0029665116000665
12. Anderson JL, Asche F, Garlock T. Globalization and commoditization: The transformation of the seafood market. *J Commod Mark*. 2018;12:2-8. doi:10.1016/j.jcomm.2017.12.004
13. Naylor RL, Hardy RW, Buschmann AH, et al. A 20-year retrospective review of global aquaculture. *Nature*. 2021;591:551-563. doi:10.1038/s41586-021-03308-6
14. EUMOFA. Aquaculture data - Yearly simple tables. Accessed March 28, 2023, https://www.eumofa.eu/en/aquaculture-yearly
15. European Commission. *Special Eurobarometer 515 - EU Consumer Habits Regarding Fishery and Aquaculture Products*. 2021. doi: 10.2771/87688.
16. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. doi:10.1136/bmj.n71
17. Richard NL, Pivarnik LF. Rhode Island branding program for local seafood: Consumer perceptions, awareness, and willingness-to-pay. *J Agric Food Syst Community Dev*. 2019;9(2):13-29. doi:10.5304/jafscd.2020.092.011
18. Cantillo J, Martin JC, Roman C. A best-worst measure of attitudes toward buying seabream and seabass products: An application to the island of Gran Canaria. *Foods*. 2021;10(1):90. doi:10.3390/foods10010090
19. Bronnmann J, Hoffmann J. Consumer preferences for farmed and ecolabeled turbot: A North German perspective. *Aquac Econ Manag*. 2018;22(3):342-361. doi:10.1080/13657305.2018.1398788
20. Feucht Y, Zander K. Of earth ponds, flow-through and closed recirculation systems - German consumers' understanding of sustainable aquaculture and its communication. *Aquaculture*. 2015;438:151-158. doi:10.1016/j.aquaculture.2015.01.005
21. Hoerterer C, Petereit J, Krause G. Informed choice: The role of knowledge in the willingness to consume aquaculture products of different groups in Germany. *Aquaculture*. 2022;556:738319. doi:10.1016/j.aquaculture.2022.738319
22. López-Mas L, Claret A, Reinders MJ, Banovic M, Krystallis A, Guerrero L. Farmed or wild fish? Segmenting European consumers based on their beliefs. *Aquaculture*. 2021;532:735992. doi:10.1016/j.aquaculture.2020.735992

23. Mitra S, Khatun MN, Prodhan MMH, Khan MA. Consumer preference, willingness to pay, and market price of capture and culture fish: Do their attributes matter? *Aquaculture*. 2021;544:737139. doi:10.1016/j.aquaculture.2021.737139
24. Olesen I, Alfnes F, Røra MB, Kolstad K. Eliciting consumers' willingness to pay for organic and welfare-labelled salmon in a non-hypothetical choice experiment. *Livest Sci*. 2010;127(2-3):218-226. doi:10.1016/j.livsci.2009.10.001
25. Peavey S, Work T, Riley J. Consumer attitudes toward fresh and frozen fish. *J Aquat Food Prod Technol*. 1994;3(2):71-87. doi:10.1300/J030v03n02_07
26. Reig L, Escobar C, Carrassón M, et al. Aquaculture perceptions in the Barcelona metropolitan area from fish and seafood wholesalers, fishmongers, and consumers. *Aquaculture*. 2019;510:256-266. doi:10.1016/j.aquaculture.2019.05.066
27. Roheim CA, Sudhakaran PO, Durham CA. Certification of shrimp and salmon for best aquaculture practices: Assessing consumer preferences in Rhode Island. *Aquac Econ Manag*. 2012;16(3):266-286. doi:10.1080/13657305.2012.713075
28. Claret A, Guerrero L, Ginés R, et al. Consumer beliefs regarding farmed versus wild fish. *Appetite*. 2014;79:25-31. doi:10.1016/j.appet.2014.03.031
29. Ruiz-Chico J, Biedma-Ferrer JM, Pea-Snchez AR, Jimnez-Garca M. Acceptance of aquaculture as compared with traditional fishing in the province of Cadiz (Spain): an empirical study from the standpoint of social carrying capacity. *Rev Aquac*. 2020;12(4):2429-2445. doi:10.1111/raq.12442
30. Ruiz-Chico J, Biedma-Ferrer JM, Peña-Sánchez AR, Jiménez-García M. Social acceptance of aquaculture in Spain: An instrument to achieve sustainability for society. *J Environ Res Public Health*. 2020;17(18):1-12. doi:10.3390/ijerph17186628
31. Ruiz-Chico J, Peña-Sánchez AR, Biedma-Ferrer JM, Jiménez-García M. Social acceptance of aquaculture in andalusian atlantic coast (Spain): An emerging economy sector. *Foods*. 2020;9(7):910. doi:10.3390/foods9070910
32. Solgaard HS, Yang Y. Consumers' perception of farmed fish and willingness to pay for fish welfare. *Br Food J*. 2011;113(8):997-1010. doi:10.1108/00070701111153751
33. Suarez-Caceres GP, Fernandez-Cabanas VM, Lobillo-Eguibar J, Perez-Urrestarazu L. Consumers' knowledge, attitudes and willingness to pay for aquaponic products in Spain and Latin America. *Int J Gastron Food Sci*. 2021;24:100350. doi:10.1016/j.ijgfs.2021.100350
34. Wongprawmas R, Sogari G, Gai F, Parisi G, Menozzi D, Mora C. How information influences consumers' perception and purchasing intention for farmed

and wild fish. *Aquaculture*. 2022;547:737504. doi:10.1016/j.aquaculture.2021.737504
35. Zander K, Feucht Y. Consumers’ willingness to pay for sustainable seafood made in Europe. *J Int Food Agribus Mark*. 2018;30(3):251-275. doi:10.1080/08974438.2017.1413611
36. Rudd MA, Pelletier N, Tyedmers P. Preferences for health and environmental attributes of farmed salmon amongst southern ontario salmon consumers. *Aquac Econ Manag*. 2011;15(1):18-45. doi:10.1080/13657305.2011.549405
37. Saidi A, Sacchi G, Cavallo C, et al. Drivers of fish choice: an exploratory analysis in Mediterranean countries. *Agric Food Econ*. 2022;10(1):29. doi:10.1186/s40100-022-00237-4
38. Yeşilsu AF, Özyurt G, Dağtekin M, Alp-Erbay E. Wild fish vs. Farmed fish: Consumer perception in Turkey. *Aquac Stud*. 2019;19(1):37-43. doi:10.4194/2618-6381-v19_1_04
39. Hoque MZ, Akhter N, Chowdhury MSR. Consumers' preferences for the traceability information of seafood safety. *Foods*. 2022;11(12):1675. doi:10.3390/foods11121675
40. Lulijwa R, Rupia EJ, Alfaro AC. Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. *Rev Aquac*. 2020;12(2):640-663. doi:10.1111/raq.12344
41. European Union. Regulation (EU) 2019/4 on the manufacture, placing on the market and use of medicated feed. 2019.
42. U.S. Food & Drug Administration. Approved Aquaculture Drugs. 2023.
43. Fernández-Polanco J, Loose SM, Luna L. Are retailers' preferences for seafood attributes predictive for consumer wants? Results from a choice experiment for seabream (*Sparus aurata*). *Aquac Econ Manag*. 2013;17(2):103-122. doi:10.1080/13657305.2013.772262
44. Milicic V, Thorarinsdottir R, Dos Santos M, Hancic MT. Commercial aquaponics approaching the European market: To consumers' perceptions of aquaponics products in Europe. *Water*. 2017;9(2):80. doi:10.3390/w9020080
45. Hossain KZ, Xue J, Rabbany MG. Consumers’ willingness to pay (WTP) for HACCP certified frozen farmed fish: A consumer survey from wet markets in Dhaka, Bangladesh. *Aquac Econ Manag*. 2022;27(1):143-158. doi:10.1080/13657305.2022.2046204
46. Tran N, Shikuku KM, Hoffmann V, et al. Are consumers in developing countries willing to pay for aquaculture food safety certification? Evidence from a field

experiment in Nigeria. *Aquaculture*. 2022;550:737829. doi:10.1016/j.aquaculture.2021.737829

47. Krešić G, Dujmić E, Lončarić D, Zrnčić S, Liović N, Pleadin J. Profiling of Croatian consumers based on their intention to consume farmed fish. *Foods*. 2022;11(14):2158. doi:10.3390/foods11142158
48. Claret A, Guerrero L, Gartzia I, Garcia-Quiroga M, Ginés R. Does information affect consumer liking of farmed and wild fish? *Aquaculture*. 2016;454:157-162. doi:10.1016/j.aquaculture.2015.12.024
49. de Moura AP, Cunha LM, Castro-Cunha M, Lima RC. A comparative evaluation of women's perceptions and importance of sustainability in fish consumption: An exploratory study among light consumers with different education levels. *Manag Environ Qual*. 2012;23(4):451-461. doi:10.1108/14777831211232263
50. Schlag AK, Ystgaard K. Europeans and aquaculture: Perceived differences between wild and farmed fish. *Br Food J*. 2013;115(2):209-222. doi:10.1108/00070701311302195
51. Allegro R, Calagna A, Lo Monaco D, et al. The assessment of the attitude of Sicilian consumers towards wild and farmed seafood products – a sample survey. *Br Food J*. 2021;123(7):2506-2536. doi:10.1108/BFJ-09-2020-0853
52. Vanhonacker F, Altintzoglou T, Luten J, Verbeke W. Does fish origin matter to European consumers?: Insights from a consumer survey in Belgium, Norway and Spain. *Br Food J*. 2011;113(4):535-549. doi:10.1108/00070701111124005
53. Verbeke W, Sioen I, Brunsø K, Henauw S, Camp J. Consumer perception versus scientific evidence of farmed and wild fish: Exploratory insights from Belgium. *Aquac Int*. 2007;15(2):121-136. doi:10.1007/s10499-007-9072-7
54. Fernández-Polanco J, Luna L. Analysis of perceptions of quality of wild and cultured seabream in Spain. *Aquac Econ Manag*. 2010;14(1):43-62. doi:10.1080/13657300903566878
55. Stefani G, Scarpa R, Cavicchi A. Exploring consumer's preferences for farmed sea bream. *Aquac Int*. 2012;20(4):673-691. doi:10.1007/s10499-011-9495-z
56. Altintzoglou T, Verbeke W, Vanhonacker F, Luten J. The image of fish from aquaculture among Europeans: Impact of exposure to balanced information. *J Aquat Food Prod Technol*. 2010;19(2):103-119. doi:10.1080/10498850.2010.492093
57. Banovic M, Reinders MJ, Claret A, Guerrero L, Krystallis A. “One Fish, Two Fish, Red Fish, Blue Fish”: How ethical beliefs influence consumer perceptions of “blue” aquaculture products? *Food Qual Prefer*. 2019;77:147-158. doi:10.1016/j.foodqual.2019.05.013

58. Kitano S, Yamamoto N. The role of consumer knowledge, experience,and heterogeneity in fish consumption: Policy lessons from Japan. *J Retail Consum Serv*. 2020;56:102151. doi:10.1016/j.jretconser.2020.102151
59. Nguyen L, Gao ZF, Anderson JL. Perception shifts in seafood consumption in the United States. *Mar Policy*. 2023;148:105438. doi:10.1016/j.marpol.2022.105438
60. Runge KK, Shaw BR, Witzling L, Hartleb C, Yang S, Peroff DM. Social license and consumer perceptions of farm-raised fish. *Aquaculture*. 2021;530:735920. doi:10.1016/j.aquaculture.2020.735920
61. Hoque MZ, Myrland Ø. Consumer preference for fish safety inspection in Bangladesh. *Aquaculture*. 2022;551:737911. doi:10.1016/j.aquaculture.2022.737911
62. Zheng QJ, Wang HH, Shogren JF. Fishing or aquaculture? Chinese consumers' stated preference for the growing environment of salmon through a choice experiment and the consequentiality effect. *Mar Resour Econ*. 2021;36(1):23-42. doi:10.1086/711385
63. Arvanitoyannis IS, Krystallis A, Panagiotaki P, Theodorou AJ. A marketing survey on Greek consumers' attitudes towards fish. *Aquac Int*. 2004;12(3):259-279. doi:10.1023/B:AQUI.0000036137.29397.12
64. Rickertsen K, Alfnes F, Combris P, Enderli G, Issanchou S, Shogren JF. French consumers' attitudes and preferences toward wild and farmed fish. *Mar Resour Econ*. 2017;32(1):59-81. doi:10.1086/689202
65. Zheng Q, Nayga RM, Yang W, Tokunaga K. Do U.S. consumers value genetically modified farmed salmon? *Food Qual Prefer*. 2023;107:104841. doi:10.1016/j.foodqual.2023.104841
66. Dey MM, Rab MA, Jahan KM, Nisapa A, Kumar A, Ahmed M. Food Safety standards and regulatory measures: Implications for selected fish exporting Asian countries. *Aquac Econ Manag*. 2005;9(1-2):217-236. doi:10.1080/13657300590967450
67. Grunert KG. Food quality and safety: consumer perception and demand. *Eur Rev Agric Econ*. 2005;32(3):369-391. doi:10.1093/eurrag/jbi011
68. Meng T, Wang C, Florkowski WJ, Yang Z. Determinants of urban consumer expenditure on aquatic products in Shanghai, China. *Aquac Econ Manag*. 2021;27(1):1-24. doi:10.1080/13657305.2021.1996480
69. Zander K, Risius A, Feucht Y, Janssen M, Hamm U. Sustainable aquaculture products: Implications of consumer awareness and of consumer preferences for promising market communication in Germany. *J Aquat Food Prod Technol*. 2018;27(1):5-20. doi:10.1080/10498850.2017.1390028

70. Young JA, Little DC, Watterson A, et al. Growing Green: The emergent role of Non-Tilapia attributes in marketing Tilapia. *Aquac Econ Manag*. 2010;14(1):63-79. doi:10.1080/13657300903566886
71. Masi M, Di Pasquale J, Vecchio Y, Pauselli G, Tribilustova E, Adinolfi F. A cross-sectional study in Mediterranean European countries to support stakeholders in addressing future market demands: Consumption of farmed fish products. *Aquac Rep*. 2022;24:101133. doi:10.1016/j.aqrep.2022.101133
72. Murray G, Wolff K, Patterson M. Why eat fish? Factors influencing seafood consumer choices in British Columbia, Canada. *Ocean Coast Manag*. 2017;144:16-22. doi:10.1016/j.ocecoaman.2017.04.007
73. Mehar M, Mekkawy W, McDougall C, Benzie JAH. Preferences for rohu fish (*L. rohita*) traits of women and men from farming households in Bangladesh and India. *Aquaculture*. 2022;547:737480. doi:10.1016/j.aquaculture.2021.737480
74. Pereira LN, Mendes JC, Mendes JP. A marketing strategy for a new functional fish. *Aquac Econ Manag*. 2017;21(3):334-354. doi:10.1080/13657305.2016.1185197
75. Witter A, Murray G, Sumaila UR. Consumer seafood preferences related to alternative food networks and their value chains. *Mar Policy*. 2021;131:104694. doi:10.1016/j.marpol.2021.104694
76. Hossain A. Quantity or quality of fish in a developing country: A hedonic analysis. *J Appl Aquac*. 2023;35(2):394-409. doi:10.1080/10454438.2021.1970687
77. Myers JJ, Govindasamy R, Ewart JW, et al. Consumer analysis in ethnic live seafood markets in the northeast region of the United States. *J Food Prod Mark*. 2010;16(2):147-165. doi:10.1080/10454440903415477
78. Pupavac SM, Jovanovic GK, Linsak Z, Glad M, Traven L, Zezelj SP. The influence on fish and seafood consumption, and the attitudes and reasons for its consumption in the Croatian population. *Front Sustain Food Syst*. 2022;6:945186. doi:10.3389/fsufs.2022.945186
79. Rosenau S, Wolgast T, Altmann B, Risius A. Consumer preference for altered color of rainbow trout (*Oncorhynchus mykiss*) fillet induced by Spirulina (*Arthrospira platensis*). *Aquaculture*. 2023;572:739522. doi:10.1016/j.aquaculture.2023.739522
80. Guney OI. Consumers' perceived differences between wild and farmed fish: A survey study in Turkey. *J Aquat Food Prod Technol*. 2019;28(2):210-220. doi:10.1080/10498850.2019.1572684

81. Tezzo X, Aung HM, Belton B, Oosterveer P, Bush SR. Consumption practices in transition: Rural-urban migration and the food fish system in Myanmar. *Geoforum*. 2021;127:33-45. doi:10.1016/j.geoforum.2021.09.013
82. Darko FA, Quagrainie KK, Chenyambuga S. Consumer preferences for farmed tilapia in Tanzania: A choice experiment analysis. *J Appl Aquac*. 2016;28(3):131-143. doi:10.1080/10454438.2016.1169965
83. Ferrer Llagostera P, Kallas Z, Reig L, Amores de Gea D. The use of insect meal as a sustainable feeding alternative in aquaculture: Current situation, Spanish consumers' perceptions and willingness to pay. *J Clean Prod*. 2019;229:10-21. doi:10.1016/j.jclepro.2019.05.012
84. Ribeiro AR, Altintzoglou T, Mendes J, Nunes ML, Dinis MT, Dias J. Farmed fish as a functional food: Perception of fish fortification and the influence of origin – Insights from Portugal. *Aquaculture*. 2019;501:22-31. doi:10.1016/j.aquaculture.2018.11.002
85. Davidson K, Pan ML, Hu WY, Poerwanto D. Consumers' willingness to pay for aquaculture fish products vs. wild-caught seafood - a case study in Hawaii. *Aquac Econ Manag*. 2012;16(2):136-154. doi:10.1080/13657305.2012.678554
86. Kralik B, Weisstein F, Meyer J, Neves K, Anderson D, Kershaw J. From water to table: A multidisciplinary approach comparing fish from aquaponics with traditional production methods. *Aquaculture*. 2022;552:737953. doi:10.1016/j.aquaculture.2022.737953
87. Li D, Ramaswamy S, Tudor KW, Spaulding AD. The North Central aquaculture region as a potential market for farm-raised hybrid striped bass. *Aquac Econ Manag*. 2008;12(2):107-129. doi:10.1080/13657300802110752
88. Yip W, Knowler D, Haider W, Trenholm R. Valuing the willingness-to-pay for sustainable seafood: integrated multitrophic versus closed containment aquaculture. *Can J Agric Econ*. 2017;65(1):93-117. doi:10.1111/cjag.12102
89. Beyer L, Chan C, LaPorte P, Lee CS. Assessing high school students' perceptions and preferences for aquaculture versus wild-caught seafood. *J World Aquac Soc*. 2023;54(4):801-813. doi:10.1111/jwas.12949
90. Gaviglio A, Demartini E. Consumer attitudes towards farm-raised and wild-caught fish: variables of product perception. *New Medit*. 2009;8(3):34-40.
91. Kole APW, Altintzoglou T, Schelvis-Smit RAAM, Luten JB. The effects of different types of product information on the consumer product evaluation for fresh cod in real life settings. *Food Qual Prefer*. 2009;20(3):187-194. doi:10.1016/j.foodqual.2008.09.003

92. Menozzi D, Wongprawmas R, Sogari G, Gai F, Parisi G, Mora C. The role of objective and subjective knowledge on the attitude and intention of Italian consumers to purchase farmed and wild fish. *Agric Food Econ*. 2023;11(1)doi:10.1186/s40100-023-00288-1
93. Sagun OK, Sayğı H. Consumption of fishery products in Turkey's coastal regions. *Br Food J*. 2021;123(9):3070-3084. doi:10.1108/BFJ-05-2020-0442
94. Vanhonacker F, Pieniak Z, Verbeke W. European consumer image of farmed fish, wild fish, seabass and seabream. *Aquac Int*. 2013;21(5):1017-1033. doi:10.1007/s10499-012-9609-2
95. Flaherty M, Reid G, Chopin T, Latham E. Public attitudes towards marine aquaculture in Canada: insights from the Pacific and Atlantic coasts. *Aquac Int*. 2019;27(1):9-32. doi:10.1007/s10499-018-0312-9
96. Alam MA, Alfnes F. Consumer preferences for fish attributes in Bangladesh: A choice experiment. *J Int Food Agribus Mark*. 2020;32(5):425-440. doi:10.1080/08974438.2019.1697409
97. Menozzi D, Nguyen TT, Sogari G, et al. Consumers' preferences and willingness to pay for fish products with health and environmental labels: Evidence from five European countries. *Nutrients*. 2020;12(9):2650. doi:10.3390/nu12092650
98. Budhathoki M, Zolner A, Nielsen T, Reinbach HC. The role of production method information on sensory perception of smoked salmon – A mixed-method study from Denmark. *Food Qual Prefer*. 2021;94:104325. doi:10.1016/j.foodqual.2021.104325
99. Drake SL, Drake MA, Daniels HV, Yates MD. Sensory properties of wild and aquacultured southern flounder (*Paralichthys lethostigma*). *J Sens Stud*. 2006;21(2):218-227. doi:10.1111/j.1745-459X.2006.00062.x
100. Lawley M, Birch D, Hamblin D. An exploratory study into the role and interplay of intrinsic and extrinsic cues in Australian consumers' evaluations of fish. *Australas Mark J*. 2012;20(4):260-267. doi:10.1016/j.ausmj.2012.05.014
101. Koral S, Tufan B, Başçınar N, Köse S. Quality changes and shelf-life of cultured and wild hot-smoked mediterranean horse mackerel (*Trachurus mediterraneus*, Steindachner, 1868) at frozen storage (-20±2°C). *Turk J Fish Aquat Sci*. 2015;15(2):495-504. doi:10.4194/1303-2712-v15_2_37
102. Antão-Geraldes AM, Hungulo SR, Pereira E, Teixeira A, Teixeira A, Rodrigues S. Body composition and sensory quality of wild and farmed brown-trout (*Salmo trutta*) and of farmed rainbow-trout (*Oncorhynchus mykiss*). *Cienc Rural*. 2018;48(9):190. doi:10.1590/0103-8478cr20180190

103. Antão-Geraldes AM, Hungulo SR, Pereira E, Teixeira A, Teixeira A, Rodrigues S. Sensory quality and consumers' acceptance towards wild and farmed brown-trout (*Salmo trutta*) and farmed rainbow-trout (*Oncorhynchus mykiss*). *Braz J Food Tech*. 2020;23:10419. doi:10.1590/1981-6723.10419
104. Pohar J. Detection and comparison of the sensory quality of wild and farmed brown trout (*Salmo trutta*) by consumers. *Acta Agricult Slov*. 2011;98(1):45-50. doi:10.14720/aas.2011.98.1.14596
105. Guy J, Nottingham S. Fillet yield, biochemical composition, and consumer acceptance of farmed and wild mulloway. *J Aquat Food Prod Technol*. 2014;23(6):608-620. doi:10.1080/10498850.2012.750636
106. Sylvia G, Morrissey MT, Graham T, Garcia S. Organoleptic qualities of farmed and wild salmon. *J Aquat Food Prod Technol*. 1995;4(1):51-64. doi:10.1300/J030v04n01_04
107. Cunha LM, Cabral D, Moura AP, de Almeida MDV. Application of the Food Choice Questionnaire across cultures: Systematic review of cross-cultural and single country studies. *Food Qual Prefer*. 2018;64:21-36. doi:10.1016/j.foodqual.2017.10.007
108. Carrassón M, Soler-Membrives A, Constenla M, et al. Information impact on consumers' perceptions towards aquaculture: Dismantling the myth about feeds for farmed fish. *Aquaculture*. 2021;544:737137. doi:10.1016/j.aquaculture.2021.737137
109. Alexander KA, Freeman S, Potts T. Navigating uncertain waters: European public perceptions of integrated multi trophic aquaculture (IMTA). *Environ Sci Policy*. 2016;61:230-237. doi:10.1016/j.envsci.2016.04.020
110. Risius A, Hamm U, Janssen M. Target groups for fish from aquaculture: Consumer segmentation based on sustainability attributes and country of origin. *Aquaculture*. 2019;499:341-347. doi:10.1016/j.aquaculture.2018.09.044
111. Risius A, Janssen M, Hamm U. Consumer preferences for sustainable aquaculture products: Evidence from in-depth interviews, think aloud protocols and choice experiments. *Appetite*. 2017;113:246-254. doi:10.1016/j.appet.2017.02.021
112. Ankamah-Yeboah I, Jacobsen JB, Olsen SB. Innovating out of the fishmeal trap: The role of insect-based fish feed in consumers' preferences for fish attributes. *Br Food J*. 2018;120(10):2395-2410. doi:10.1108/BFJ-11-2017-0604
113. Budhathoki M, Zolner A, Nielsen T, Rasmussen MA, Reinbach HC. Intention to buy organic fish among Danish consumers: Application of the segmentation

approach and the theory of planned behaviour. *Aquaculture*. 2022;549:737798. doi:10.1016/j.aquaculture.2021.737798

114. van Osch S, Hynes S, O'Higgins T, Hanley N, Campbell D, Freeman S. Estimating the Irish public's willingness to pay for more sustainable salmon produced by integrated multi-trophic aquaculture. *Mar Policy*. 2017;84:220-227. doi:10.1016/j.marpol.2017.07.005
115. Bimbo F, Viscecchia R, De Devitiis B, Seccia A, Roma R, De Boni A. How do Italian consumers value sustainable certifications on fish? – An explorative analysis. *Sustainability*. 2022;14(6):3654. doi:10.3390/su14063654
116. Claret A, Guerrero L, Aguirre E, et al. Consumer preferences for sea fish using conjoint analysis: Exploratory study of the importance of country of origin, obtaining method, storage conditions and purchasing price. *Food Qual Prefer*. 2012;26(2):259-266. doi:10.1016/j.foodqual.2012.05.006
117. Sigurdsson V, Larsen NM, Pálsdóttir RG, Folwarczny M, Menon RGV, Fagerstrøm A. Increasing the effectiveness of ecological food signaling: Comparing sustainability tags with eco-labels. *J Bus Res*. 2022;139:1099-1110. doi:10.1016/j.jbusres.2021.10.052
118. van Osch S, Hynes S, Freeman S, O'Higgins T. Estimating the public's preferences for sustainable aquaculture: A country comparison. *Sustainability*. 2019;11(3):569. doi:10.3390/su11030569
119. Banovic M, Reinders MJ, Claret A, Guerrero L, Krystallis A. A cross-cultural perspective on impact of health and nutrition claims, country-of-origin and eco-label on consumer choice of new aquaculture products. *Food Res Int*. 2019;123:36-47. doi:10.1016/j.foodres.2019.04.031
120. Reinders MJ, Banović M, Guerrero L, Krystallis A. Consumer perceptions of farmed fish: A cross-national segmentation in five European countries. *Br Food J*. 2016;118(10):2581-2597. doi:10.1108/BFJ-03-2016-0097
121. Güney OI, Özsahinoglu I, Erçen Z, Yeldan H, Dikel C, Sangün L. The mediator role of attitudes in fish choice behavior: A Turkish market survey. *Foods*. 2022;11(20):3180. doi:10.3390/foods11203180
122. Pulcini D, Franceschini S, Buttazzoni L, Giannetti C, Capoccioni F. Consumer preferences for farmed seafood: An Italian case study. *J Aquat Food Prod Technol*. 2020;29(5):445-460. doi:10.1080/10498850.2020.1749201
123. Short G, Yue C, Anderson N, Russell C, Phelps N. Consumer perceptions of aquaponic systems. *HortTechnology*. 2017;27(3):358-366. doi:10.21273/HORTTECH03606-16

124. Regnier E, Bayramoglu B. Competition between farmed and wild fish: The French sea bass and sea bream markets. *Aquac Econ Manag*. 2017;21(3):355-375. doi:10.1080/13657305.2016.1189012
125. Rodríguez GR, Bande R, Villasante S. Origins matter: (no) market integration between cultured and wild gilthead sea bream in the spanish seafood market. *Aquac Econ Manag*. 2013;17(4):380-397. doi:10.1080/13657305.2013.825932
126. Bronnmann J, Asche F. The value of product attributes, brands and private labels: An analysis of frozen seafood in Germany. *J Agric Econ*. 2016;67(1):231-244. doi:10.1111/1477-9552.12138
127. Bronnmann J, Loy JP, Schroeder KJ. Characteristics of demand structure and preferences for wild and farmed seafood in Germany: An application of QUAIDS modeling with correction for sample selection. *Mar Resour Econ*. 2016;31(3):281-300. doi:10.1086/686692
128. Genschick S, Marinda P, Tembo G, Kaminski AM, Thilsted SH. Fish consumption in urban Lusaka: The need for aquaculture to improve targeting of the poor. *Aquaculture*. 2018;492:280-289. doi:10.1016/j.aquaculture.2018.03.052
129. Kalshoven K, Meijboom FLB. Sustainability at the crossroads of fish consumption and production ethical dilemmas of fish buyers at retail organizations in The Netherlands. *J Agric Environ Ethics*. 2013;26(1):101-117. doi:10.1007/s10806-011-9367-6
130. Ares G, de Saldamando L, Giménez A, et al. Consumers' associations with wellbeing in a food-related context: A cross-cultural study. *Food Qual Prefer*. 2015;40:304-315. doi:10.1016/j.foodqual.2014.06.001
131. Kosti RI, Kasdagli MI, Kyrozis A, et al. Fish intake, n-3 fatty acid body status, and risk of cognitive decline: a systematic review and a dose-response meta-analysis of observational and experimental studies. *Nutr Rev*. 2022;80(6):1445-1458. doi:10.1093/nutrit/nuab078
132. Cipolari OC, de Oliveira Neto XA, Conceição K. Fish bioactive peptides: A systematic review focused on sting and skin. *Aquaculture*. 2020;515:734598. doi:10.1016/j.aquaculture.2019.734598
133. Carlucci D, Nocella G, De Devitiis B, Viscecchia R, Bimbo F, Nardone G. Consumer purchasing behaviour towards fish and seafood products. Patterns and insights from a sample of international studies. *Appetite*. 2015;84:212-227. doi:10.1016/j.appet.2014.10.008
134. Verbeke W, Vanhonacker F, Sioen I, Van Camp J, De Henauw S. Perceived importance of sustainability and ethics related to fish: A consumer behavior

perspective. *Ambio*. 2007;36(7):580-585. doi:10.1579/0044-7447(2007)36[580:Piosae]2.0.Co;2

135. Altintzoglou T, Vanhonacker F, Verbeke W, Luten J. Association of health involvement and attitudes towards eating fish on farmed and wild fish consumption in Belgium, Norway and Spain. *Aquac Int*. 2011;19(3):475-488. doi:10.1007/s10499-010-9363-2
136. Mairesse G, Thomas M, Gardeur J-N, Brun-Bellut J. Effects of geographic source, rearing system, and season on the nutritional quality of wild and farmed *Perca fluviatilis*. *Lipids*. 2006;41(3):221-229. doi:10.1007/s11745-006-5091-9
137. Sales J. Quantification of the differences in flesh fatty acid components between farmed and wild fish. *J Aquat Food Prod Technol*. 2010;19(3-4):298-309. doi:10.1080/10498850.2010.519861
138. Grigorakis K. Compositional and organoleptic quality of farmed and wild gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) and factors affecting it: A review. *Aquaculture*. 2007;272(1):55-75. doi:10.1016/j.aquaculture.2007.04.062
139. Manthey-Karl M, Lehmann I, Ostermeyer U, Schröder U. Natural chemical composition of commercial fish species: Characterisation of pangasius, wild and farmed turbot and barramundi. *Foods*. 2016;5(3):58. doi:10.3390/foods5030058
140. Oztekin A, Yigit M, Kizilkaya B, et al. Nutritional quality of amino acid in farmed, farm-aggregated and wild axillary seabream (*Pagellus acarne*) with implications to human health. *Aquac Res*. 2020;51(5):1844-1853. doi:10.1111/are.14534
141. Henriques J, Dick JR, Tocher DR, Bell JG. Nutritional quality of salmon products available from major retailers in the UK: Content and composition of n-3 long-chain PUFA. *Br J Nutr*. 2014;112(6):964-975. doi:10.1017/S0007114514001603
142. Modzelewska-Kapituła M, Pietrzak-Fiećko R, Kozłowski K, Szczepkowski M, Zakęś Z. Muscle tissue quality of raw and sous-vide cooked wild and farmed pikeperch. *Foods*. 2022;11(23):3811. doi:10.3390/foods11233811
143. Parma L, Badiani A, Bonaldo A, et al. Farmed and wild common sole (*Solea solea* L.): Comparative assessment of morphometric parameters, processing yields, selected nutritional traits and sensory profile. *Aquaculture*. 2019;502:63-71. doi:10.1016/j.aquaculture.2018.12.029
144. Al-Asheeri S, Freije A, Perna S. Farmed versus wild fish consumption in relation to fatty acid composition in the Kingdom of Bahrain. *Egypt J Aquat Biol Fish*. 2020;24(7- Special issue):803-816. doi:10.21608/ejabf.2020.151489
145. Jiang H, Cheng X, Geng L, Tang S, Tong G, Xu W. Comparative study of the nutritional composition and toxic elements of farmed and wild *Chanodichthys*

mongolicus. *Chin J Oceanol Limnol*. 2017;35(4):737-744. doi:10.1007/s00343-017-6119-0
146. O'Neill B, Le Roux A, Hoffman LC. Comparative study of the nutritional composition of wild versus farmed yellowtail (*Seriola lalandi*). *Aquaculture*. 2015;448:169-175. doi:10.1016/j.aquaculture.2015.05.034
147. Hall TE, Amberg SM. Factors influencing consumption of farmed seafood products in the Pacific northwest. *Appetite*. 2013;66:1-9. doi:10.1016/j.appet.2013.02.012
148. Grunert KG, Wills JM, Fernández-Celemín L. Nutrition knowledge, and use and understanding of nutrition information on food labels among consumers in the UK. *Appetite*. 2010;55(2):177-189. doi:10.1016/j.appet.2010.05.045
149. Strijbos C, Schluck M, Bisschop J, et al. Consumer awareness and credibility factors of health claims on innovative meat products in a cross-sectional population study in the Netherlands. *Food Qual Prefer*. 2016;54:13-22. doi:10.1016/j.foodqual.2016.06.014
150. Žeželj I, Milošević J, Stojanović Ž, Ognjanov G. The motivational and informational basis of attitudes toward foods with health claims. *Appetite*. 2012;59(3):960-967. doi:10.1016/j.appet.2012.09.008
151. Román S, Sánchez-Siles LM, Siegrist M. The importance of food naturalness for consumers: Results of a systematic review. *Trends Food Sci Tech*. 2017;67:44-57. doi:10.1016/j.tifs.2017.06.010
152. Giordano S, Clodoveo ML, Gennaro BD, Corbo F. Factors determining neophobia and neophilia with regard to new technologies applied to the food sector: A systematic review. *Int J Gastron Food Sci*. 2018;11:1-19. doi:10.1016/j.ijgfs.2017.10.001
153. Siegrist M. Factors influencing public acceptance of innovative food technologies and products. *Trends Food Sci Tech*. 2008;19(11):603-608. doi:10.1016/j.tifs.2008.01.017
154. Cardello AV, Schutz HG, Lesher LL. Consumer perceptions of foods processed by innovative and emerging technologies: A conjoint analytic study. *Innov Food Sci Emmerg Technol*. 2007;8(1):73-83. doi:10.1016/j.ifset.2006.07.002
155. Cantillo J, Martín JC, Román C. Understanding consumers perceptions of aquaculture and its products in Gran Canaria island: Does the influence of positive or negative wording matter? *Aquaculture*. 2023;562:738754. doi:10.1016/j.aquaculture.2022.738754

156. Evans G, de Challemaison B, Cox DN. Consumers' ratings of the natural and unnatural qualities of foods. *Appetite*. 2010;54(3):557-563. doi:10.1016/j.appet.2010.02.014
157. Ahmed N, Turchini GM. Recirculating aquaculture systems (RAS): Environmental solution and climate change adaptation. *J Clean Prod*. 2021;297:126604. doi:10.1016/j.jclepro.2021.126604
158. Yue K, Shen Y. An overview of disruptive technologies for aquaculture. *Aquac Fish*. 2022;7(2):111-120. doi:10.1016/j.aaf.2021.04.009
159. European Market Observatory for Fisheries and Aquaculture Products. *Recirculating Aquaculture Systems*. 2020. doi: 10.2771/66025.
160. Rickard LN, Houston CL, McGreavy B, Johnson BB, Gurney G. Fish prisons and bluehouses: Perceived risks and benefits of land-based aquaculture in four US communities. *Environ Commun*. 2022:1-17. doi:10.1080/17524032.2022.2062020
161. Fong CR, Gonzales CM, Rennick M, et al. Conflict and alignment on aquaculture among Californian communities. *Aquaculture*. 2023;580doi:10.1016/j.aquaculture.2023.740230
162. Cho Y-N. Different shades of green consciousness: The interplay of sustainability labeling and environmental impact on product evaluations. *J Bus Ethics*. 2015;128(1):73-82. doi:10.1007/s10551-014-2080-4
163. Whitmarsh D, Palmieri MG. Consumer behaviour and environmental preferences: A case study of Scottish salmon aquaculture. *Aquac Res*. 2011;42(SUPPL. 1):142-147. doi:10.1111/j.1365-2109.2010.02672.x
164. Forleo MB, Romagnoli L, Palmieri N. Are Italian consumers of canned tuna fish sensitive to environmentally sustainable product attributes? *Br Food J*. 2023;125(2):608-625. doi:10.1108/bfj-08-2021-0893
165. Hoque MZ. Sustainability indicators for sustainably-farmed fish in Bangladesh. *Sustain Prod Consum*. 2021;27:115-127. doi:10.1016/j.spc.2020.10.020
166. Hynes S, Ravagnan E, Gjerstad B. Do concerns for the environmental credentials of salmon aquaculture translate into WTP a price premium for sustainably farmed fish? A contingent valuation study in Ireland and Norway. *Aquac Int*. 2019;27(6):1709-1723. doi:10.1007/s10499-019-00425-y
167. Hilger J, Hallstein E, Stevens AW, Villas-Boas SB. Measuring willingness to pay for environmental attributes in seafood. *Environ Resour Econ*. 2019;73(1):307-332. doi:10.1007/s10640-018-0264-6

168. Freeman S, Vigoda-Gadot E, Sterr H, et al. Public attitudes towards marine aquaculture: A comparative analysis of Germany and Israel. *Environ Sci Policy*. 2012;22:60-72. doi:10.1016/j.envsci.2012.05.004
169. Hinkes C, Schulze-Ehlers B. Consumer attitudes and preferences towards pangasius and tilapia: The role of sustainability certification and the country of origin. *Appetite*. 2018;127:171-181. doi:10.1016/j.appet.2018.05.001
170. Jonell M, Crona B, Brown K, Rönnbäck P, Troell M. Eco-labeled seafood: Determinants for (blue) green consumption. *Sustainability*. 2016;8(9)doi:10.3390/su8090884
171. Olsen MS, Amundsen VS, Osmundsen TC. Exploring public perceptions and expectations of the salmon aquaculture industry in Norway: A social license to operate? *Aquaculture*. 2023;574:739632. doi:10.1016/j.aquaculture.2023.739632
172. Longstaff HA. Frankenfish, monsatan, and killer canola making good decisions in the midst of environmental controversy. *J Environ Inform*. 2005;6(1):33-45. doi:10.3808/jei.200500053
173. Forleo MB, Palmieri N. Environmental attributes of wild versus farmed tuna: Beliefs, knowledge and purchasing choices of Italian consumers of canned tuna. *Sustainability*. 2023;15(9):7149. doi:10.3390/su15097149
174. López-Mas L, Claret A, Arvisenet G, et al. European consumers' beliefs about the main pillars of the sustainability: a comparison between wild and farmed fish. *Aquac Int*. 2023;31(4):2035-2055. doi:10.1007/s10499-023-01070-2
175. Yi S. Determinants of consumers' purchasing behavior for certified aquaculture products in South Korea. *Sustainability*. 2019;11(14):3840. doi:10.3390/su11143840
176. Gould D, Compagnoni A, Lembo G. Organic aquaculture: Principles, standards and certification. In: Lembo G, Mente E, eds. *Organic Aquaculture Impacts and Future Developments*. Springer Nature; 2019:1-22.
177. European Union. Regulation (EU) 2018/848 of the European Parlieament and of the Council of 30 May 2018 on Organic Production and Labelling of Organic Products and Repealing Council Regulation (EC) No 834/2007. 2018.
178. Ferfolja M, Cerjak M, Matulić D, Maksan MT. Consumer knowledge and perception about fresh fish from organic farming in Croatia. *Ribar Croat J Fish*. 2022;80(1):7-16. doi:10.2478/cjf-2022-0002
179. Mauracher C, Tempesta T, Vecchiato D. Consumer preferences regarding the introduction of new organic products. The case of the Mediterranean sea bass (*Dicentrarchus labrax*) in Italy. *Appetite*. 2013;63:84-91. doi:10.1016/j.appet.2012.12.009

180. Budak F, Budak DB, Kacira OO, Yavuz MC. Consumer willingness to pay for organic sea bass in Turkey. *Isr J Aquac*. 2006;58(2):116-123. doi:10.46989/001c.20431
181. Ankamah-Yeboah I, Jacobsen JB, Olsen SB, Nielsen M, Nielsen R. The impact of animal welfare and environmental information on the choice of organic fish: An empirical investigation of German trout consumers. *Mar Resour Econ*. 2019;34(3):248-266. doi:10.1086/705235
182. Ankamah-Yeboah I, Nielsen M, Nielsen R. Price premium of organic salmon in Danish retail sale. *Ecol Econ*. 2016;122:54-60. doi:10.1016/j.ecolecon.2015.11.028
183. Buck BH, Troell MF, Krause G, Angel DL, Grote B, Chopin T. State of the art and challenges for offshore Integrated Multi-Trophic Aquaculture (IMTA). *Front Mar Sci*. 2018;5:165. doi:10.3389/fmars.2018.00165
184. Knowler D, Chopin T, Martínez-Espiñeira R, et al. The economics of Integrated Multi-Trophic Aquaculture: where are we now and where do we need to go? *Rev Aquac*. 2020;12(3):1579-1594. doi:10.1111/raq.12399
185. Alexander KA, Angel D, Freeman S, et al. Improving sustainability of aquaculture in Europe: Stakeholder dialogues on Integrated Multi-trophic Aquaculture (IMTA). *Environ Sci Policy*. 2016;55:96-106. doi:10.1016/j.envsci.2015.09.006
186. Goddek S, Delaide B, Mankasingh U, Ragnarsdottir KV, Jijakli H, Thorarinsdottir R. Challenges of sustainable and commercial aquaponics. *Sustainability*. 2015;7(4):4199-4224. doi:10.3390/su7044199
187. Yi S. Contingent valuation of sustainable integrated agriculture-aquaculture products: The case of rice-fish farming systems in South Korea. *Agronomy*. 2019;9(10):601. doi:10.3390/agronomy9100601
188. Greenfeld A, Becker N, Bornman JF, dos Santos MJ, Angel D. Consumer preferences for aquaponics: A comparative analysis of Australia and Israel. *J Environ Manag*. 2020;257:10.1016/j.jenvman.2019.109979. doi:10.1016/j.jenvman.2019.109979
189. Weitzman J, Bailey M. Perceptions of aquaculture ecolabels: A multi-stakeholder approach in Nova Scotia, Canada. *Mar Policy*. 2018;87:12-22. doi:10.1016/j.marpol.2017.09.037
190. Chikudza L, Gauzente C, Guillotreau P, Alexander KA. Producer perceptions of the incentives and challenges of adopting ecolabels in the European finfish aquaculture industry: A Q-methodology approach. *Mar Policy*. 2020;121:104176. doi:10.1016/j.marpol.2020.104176

191. Petereit J, Hoerterer C, Krause G. Country-specific food culture and scientific knowledge transfer events – Do they influence the purchasing behaviour of seafood products? *Aquaculture*. 2022;560:738590. doi:10.1016/j.aquaculture.2022.738590
192. Ariji M. Conjoint analysis of consumer preference for bluefin tuna. *Fish Sci*. 2010;76(6):1023-1028. doi:10.1007/s12562-010-0297-4
193. Gulbrandsen LH, Vormedal I, Larsen ML. No logo? The failure of ASC salmon labeling in Norway and the UK. *Mar Policy*. 2022;138doi:10.1016/j.marpol.2022.104987
194. Blomquist J, Bartolino V, Waldo S. Price premiums for providing eco-labelled seafood: Evidence from MSC-certified cod in Sweden. *J Agric Econ*. 2015;66(3):690-704. doi:10.1111/1477-9552.12106
195. Bronnmann J, Asche F. Sustainable seafood from aquaculture and wild fisheries: insights from a discrete choice experiment in Germany. *Ecol Econ*. 2017;142:113-119. doi:10.1016/j.ecolecon.2017.06.005
196. Asche F, Bronnmann J, Cojocaru AL. The value of responsibly farmed fish: A hedonic price study of ASC-certified whitefish. *Ecol Econ*. 2021;188:107135. doi:10.1016/j.ecolecon.2021.107135
197. Chen X, Alfnes F, Rickertsen K. Consumer preferences, ecolabels, and effects of negative environmental information. *AgBioForum*. 2015;18(3):327-336.
198. Prem R, Tewari VK. Development of human-powered fish feeding machine for freshwater aquaculture farms of developing countries. *Aquac Eng*. 2020;88:102028. doi:10.1016/j.aquaeng.2019.102028
199. Dauda AB, Ajadi A, Tola-Fabunmi AS, Akinwole AO. Waste production in aquaculture: sources, components and managements in different culture systems. *Aquac Fish*. 2019;4(3):81-88. doi:10.1016/j.aaf.2018.10.002
200. Jannathulla R, Rajaram V, Kalanjiam R, Ambasankar K, Muralidhar M, Dayal JS. Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. *Aquac Res*. 2019;50(12):3493-3506. doi:10.1111/are.14324
201. Maulu S, Langi S, Hasimuna OJ, et al. Recent advances in the utilization of insects as an ingredient in aquafeeds: A review. *Anim Nutr*. 2022;11:334-349. doi:10.1016/j.aninu.2022.07.013
202. Ho SS, Tan WQ, Chuah ASF, Chua PYY, Lau KXL, Chan JZA. Feed for thought: Factors predicting public support for funding and labeling preferences of alternative aquafeed. *Environ Commun*. 2022:1-19. doi:10.1080/17524032.2022.2051577

203. Mancuso T, Baldi L, Gasco L. An empirical study on consumer acceptance of farmed fish fed on insect meals: the Italian case. *Aquac Int*. 2016;24(5):1489-1507. doi:10.1007/s10499-016-0007-z
204. Popoff M, MacLeod M, Leschen W. Attitudes towards the use of insect-derived materials in Scottish salmon feeds. *J Insects Food Feed*. 2017;3(2):131-138. doi:10.3920/JIFF2016.0032
205. Baldi L, Mancuso T, Peri M, Gasco L, Trentinaglia MT. Consumer attitude and acceptance toward fish fed with insects: a focus on the new generations. *J Insects Food Feed*. 2022;8(11):1249-1263. doi:10.3920/jiff2021.0109
206. Baldi L, Trentinaglia MT, Peri M, Panzone L. Nudging the acceptance of insects-fed farmed fish among mature consumers. *Aquac Econ Manag*. 2023:2265875. doi:10.1080/13657305.2023.2265875
207. Bazoche P, Poret S. Acceptability of insects in animal feed: A survey of French consumers. *J Consum Behav*. 2021;20(2):251-270. doi:10.1002/cb.1845
208. Arru B, Furesi R, Pulina P, Madau FA. Price sensitivity of fish fed with insect meal: An analysis on Italian consumers. *Sustainability*. 2022;14(11):6657. doi:10.3390/su14116657
209. Fraser D. Animal welfare and the intensification of animal production. In: Thompson PB, ed. *The Ethics of Intensification: Agricultural Development and Cultural Change*. Springer Netherlands; 2008:167-189.
210. Grimsrud KM, Nielsen HM, Navrud S, Olesen I. Households' willingness-to-pay for improved fish welfare in breeding programs for farmed Atlantic salmon. *Aquaculture*. 2013;372:19-27. doi:10.1016/j.aquaculture.2012.10.009
211. Sloman KA, Bouyoucos IA, Brooks EJ, Sneddon LU. Ethical considerations in fish research. *J Fish Biol*. 2019;94(4):556-577. doi:10.1111/jfb.13946
212. Farm Animal Welfare Committee. *Farm animal welfare in Great Britain: past, present and future*. 2009.
213. Stien LH, Bracke M, Noble C, Kristiansen TS. Assessing fish welfare in aquaculture. In: Kristiansen TS, Fernö A, Pavlidis MA, Vis Hvd, eds. *The Welfare of Fish*. Springer Nature; 2020:303-321.
214. European Union. Directive 98/58/EC of the European Parliament and of the council of 20 July 1998 concerning the protection of animals kept for farming purposes. Official Journal of the European Union; 1998.
215. Levenda K. Legislation to protect the welfare of fish. *Anim Law Rev*. 2013;119:20.

216. Seibel H, Weirup L, Schulz C. Fish welfare – Between regulations, scientific facts and human perception. *Food Ethics*. 2020;5(1-2):4. doi:10.1007/s41055-019-00063-3
217. Ellingsen K, Grimsrud K, Nielsen HM, et al. Who cares about fish welfare? A Norwegian study. *Br Food J*. 2015;117(1):257-273. doi:10.1108/bfj-08-2013-0223
218. Kupsala S, Jokinen P, Vinnari M. Who cares about farmed fish? Citizen perceptions of the welfare and the mental abilities of fish. *J Agric Environ Ethics*. 2013;26(1):119-135. doi:10.1007/s10806-011-9369-4
219. Frewer LJ, Kole A, van de Kroon SM, de Lauwere C. Consumer attitudes towards the development of animal-friendly husbandry systems. *J Agric Environ Ethics*. 2005;18(4):345-367. doi:10.1007/s10806-005-1489-2
220. Hartmann C, Siegrist M. Our daily meat: Justification, moral evaluation and willingness to substitute. *Food Qual Prefer*. 2020;80:103799. doi:10.1016/j.foodqual.2019.103799
221. Honkanen P, Olsen SO. Environmental and animal welfare issues in food choice: The case of farmed fish. *Br Food J*. 2009;111(2-3):293-309. doi:10.1108/00070700910941480
222. Kulesz MM, Lundh T, de Koning DJ, Lagerkvist CJ. Dissuasive effect, information provision, and consumer reactions to the term 'Biotechnology': The case of reproductive interventions in farmed fish. *PLoS One*. 2019;14(9):e0222494. doi:10.1371/journal.pone.0222494
223. Pieniak Z, Vanhonacker F, Verbeke W. Consumer knowledge and use of information about fish and aquaculture. *Food Policy*. 2013;40:25-30. doi:10.1016/j.foodpol.2013.01.005
224. Stancu V, Brunsø K, Krystallis A, Guerrero L, Santa Cruz E, Peral I. European consumer segments with a high potential for accepting new innovative fish products based on their food-related lifestyle. *Food Qual Prefer*. 2022;99:104560. doi:10.1016/j.foodqual.2022.104560
225. Flores RMV, Widmar NO, Quagrainie K, Preckel PV, Pedroza Filho MX. Establishing linkages between consumer fish knowledge and demand for fillet attributes in Brazilian supermarkets. *J Int Food Agribus Mark*. 2022;34(4):368-388. doi:10.1080/08974438.2021.1900016
226. Zhou GZ, Hu WY, Huang WC. Are consumers willing to pay more for sustainable products? A study of eco-labeled tuna steak. *Sustainability*. 2016;8(5):494. doi:10.3390/su8050494

227. Hoque MZ, Alam MN. Consumers' knowledge discrepancy and confusion in intent to purchase farmed fish. *Br Food J*. 2020;122(11):3567-3583. doi:10.1108/bfj-01-2019-0021
228. Abdikoglu DI, Azabagaoglu MO, Unakitan G. An econometric analysis of factors affecting fish consumption: The case of Tekirdag, Turkey. *KSU J Agric Nat*. 2020;23(2):446-452. doi:10.18016/ksutarimdoga.vi.623343
229. Hoque MZ, Sultana N, Haque A, Foisal MTM. Personal and socioeconomic factors affecting perceived knowledge of farmed fish. *J Agric Food Res*. 2022;8:100310. doi:10.1016/j.jafr.2022.100310
230. Uchida H, Roheim CA, Johnston RJ. Balancing the health risks and benefits of seafood: How does available guidance affect consumer choices? . *Am J Agric Econ*. 2017;99(4):1056-1077. doi:10.1093/ajae/aax025
231. Hansen H, Onozaka Y. When diseases hit aquaculture: An experimental study of spillover effects from negative publicity. *Mar Resour Econ*. 2011;26(4):281-291. doi:10.5950/0738-1360-26.4.281
232. Froehlich HE, Gentry RR, Rust MB, Grimm D, Halpern BS. Public perceptions of aquaculture: Evaluating spatiotemporal patterns of sentiment around the world. *PLoS One*. 2017;12(1):e0169281. doi:10.1371/journal.pone.0169281
233. Amberg SM, Hall TE. Precision and rhetoric in media reporting about contamination in farmed salmon. *Sci Commun*. 2010;32(4):489-513. doi:10.1177/1075547009357599
234. Baraibar-Diez E, Llorente I, Odriozola MD. Text emotion analysis in aquaculture communication via Twitter: The case of Spain. *Mar Policy*. 2023;152:105605. doi:10.1016/j.marpol.2023.105605
235. Bogliacino F, Charris R, Codagnone C, et al. Less is more: Information overload in the labelling of fish and aquaculture products. *Food Policy*. 2023;116:102435. doi:10.1016/j.foodpol.2023.102435
236. Yang Y, Hobbs JE, Natcher DC. Assessing consumer willingness to pay for Arctic food products. *Food Policy*. 2020;92:101846. doi:10.1016/j.foodpol.2020.101846
237. Quagrainie KK. Consumer willingness to pay for a saline fish species grown in the US midwest: The case of striped bass, *Morone saxatilis*. *J World Aquac Soc*. 2019;50(1):163-171. doi:10.1111/jwas.12464
238. Athnos A, de Souza SV, Quagrainie K, et al. Are U.S. consumers wilting to pay more by the take? An analysis of preferences for Great Lakes region fish. *Agric Resour Econ Rev*. 2022:1-26. doi:10.1017/age.2022.18

239. Garlock T, Nguyen L, Anderson J, Musumba M. Market potential for Gulf of Mexico farm-raised finfish. *Aquac Econ Manag*. 2020;24(2):128-142. doi:10.1080/13657305.2019.1691676
240. Abaidoo E, Malone T, Melstrom RT. Fish demand in the US Great Lakes region in the face of seafood mislabeling. *Aquac Econ Manag*. 2023;27(4):666-692. doi:10.1080/13657305.2023.2167019
241. Quagrainie K, Hart S, Brown P. Consumer acceptance of locally grown food: The case of Indiana aquaculture products. *Aquac Econ Manag*. 2008;12(1):54-70. doi:10.1080/13657300801962591
242. Hoque MZ, Bashar MA, Akhter F. Ambiguity tolerance and confusion avoidance in the intent to purchase farmed fish. *J Appl Aquac*. 2022;34(3):564-597. doi:10.1080/10454438.2021.1872462
243. Polymeros K, Kaimakoudi E, Schinaraki M, Batzios C. Analysing consumers' perceived differences in wild and farmed fish. *Br Food J*. 2015;117(3):1007-1016. doi:10.1108/BFJ-12-2013-0362
244. Tomić M, Lucević Z, Tomljanović T, Matulić D. Wild-caught versus farmed fish – consumer perception. *Ribar Croat J Fish*. 2017;75(2):41-50. doi:10.1515/cjf-2017-0007
245. Nash CE. *The History of Aquaculture*. Blackwell Publishing; 2011.
246. Banovic M, Krystallis A, Guerrero L, Reinders MJ. Consumers as co-creators of new product ideas: An application of projective and creative research techniques. *Food Res Int*. 2016;87:211-223. doi:10.1016/j.foodres.2016.07.010
247. Cardoso C, Lourenço H, Costa S, Gonçalves S, Nunes ML. Survey into the seafood consumption preferences and patterns in the portuguese population. Gender and regional variability. *Appetite*. 2013;64:20-31. doi:10.1016/j.appet.2012.12.022
248. Loncaric D, Dujmic E, Kresic G. Assessment of the intention to consume farmed fish using the theory of planned behaviour - Scale validation. *J Polytech Rijeka*. 2021;9(1):1-17. doi:10.31784/zvr.9.1.1
249. Cerveira I, Baptista V, Teodósio MA, Morais P. What's for dinner? Assessing the value of an edible invasive species and outreach actions to promote its consumption. *Biological Invasions*. 2022;24(3):815-829. doi:10.1007/s10530-021-02685-3
250. Cantillo J, Martin JC, Roman C. Discrete choice experiments in the analysis of consumers' preferences for finfish products: A systematic literature review. *Food Qual Prefer*. 2020;84:103952. doi:10.1016/j.foodqual.2020.103952

251. Lee MK, Nam J. The determinants of live fish consumption frequency in South Korea. *Food Res Int*. 2019;120:382-388. doi:10.1016/j.foodres.2019.03.005
252. Sajiki T, Lu YH. Japanese consumer preference for raw Fish: best-worst scaling method. *J Mar Sci Tech*. 2021;29(6):810-818. doi:10.51400/2709-6998.2560

Supplementary material of *Emerging themes on the perception of wild and farmed fish*, by Daniela Resende, Ana Pinto de Moura, Rui Costa Lima, Luisa M.P. Valente, Luís M. Cunha, to be submitted to *Reviews in Aquaculture.*

Table S1 - List of articles included in the review. Note: COO, country of origin, FF, farmed fish; WF, wild fish; WTP, willingness to pay.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
Peavey et al. 1994 [1]	New Jersey and Missouri, USA	Both	Salmon	8 per group (total 32), mix of men and women, main food shoppers	Focus groups, 2 per region, one of age 25-40 and the other 41-62.	Consumers are concerned about “chemicals” fed to fish, injections and handling. Several claimed farmed fish tasted “terrible”.
Sylvia et al. 1995 [2]	Oregon, USA	Both	Chinook and Atlantic salmon	189	Atlantic salmon was exclusively farmed, but chinook was either wild or farmed. Tests conducted at a seafood festival. Fish was tested within 72h of harvest. Steaks were cooked in a microwave oven (15 seconds per ounce). Paired blind taste tests. Hedonic preference and perceived intensity of organoleptic qualities were assessed.	Wild chinook was preferred over both farmed chinook and farmed Atlantic in overall enjoyment and delicate/fresh fish flavour.
Arvanitoyannis et al. 2004 [3]	Greece	Both	General	1093 respondents	Personal interviews. Survey: past and present preference for fish consumption; attitude towards cultivated marine fish; attitude towards fish packaging; factors inducing fish purchasing/consumption (Likert scale).	86% prefer wild to farmed. 52% believe safety of farmed fish is lower than wild. 60.7% are not aware of requirements regarding fish welfare, packaging and distribution in aquaculture. Factors affecting purchasing: prices, season, and size according to fish type. Clusters: 1) price sensitive; 2) nouveau-rich, neo-traditional; 3) inexperienced; 4) experienced.
Frewer et al. 2005 [4]	Netherlands	FF	General	1000, with quota sampling with respect to age, gender, socio-economic class, and living environment (rural versus urban)	Online survey. Half of respondents answered a survey about welfare of farmed pigs, and the other half about farmed fish. 1, Purchase criteria and knowledge of animal husbandry systems; 2, attitudes towards animal welfare; 3, sociodemographics.	Greater concern was expressed about the welfare of pigs compared to fish, as fish were perceived as being less likely to experience emotions. Consumer trust in labeling also emerged as an important issue.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
Longstaff 2005 [5]	Canada	Both	Salmon	49, involved in food purchasing at home	In-person survey. 1) multiple choice questions about genetically engineering technologies and salmon aquaculture; 2) different methods for communicating risks and benefits of using genetically engineered salmon feeds: flowchart, case study or frequently asked questions (FAQ); 3) retesting the questions and rating the communication methods.	Prior vs after info: 88% vs 98% know what salmon farming is; 45% vs 92% know what wild salmon eat; 78% vs 100% know what farmed salmon eat; 16% vs 96% know where salmon feed comes from; 67% vs 100% know what canola is and 82% vs 100% know where genetically engineered canola is grown. No differences on correct answers due to info method. Confidence about salmon aquaculture and genetically engineered products ↑ with info. Concerns about farmed salmon ↓ after info except for "harm environment". 39% said info made less likely to buy farmed salmon.
Budak et al. 2006 [6]	Adana, Turkey	FF	European seabass	253 consumers	Survey conducted in supermarkets with a fresh seafood section. 1) purchasing habits, seafood consumption, environmental/food safety concerns; 2) Consumer willingness to pay for organically farmed seabass: after information on organic aquaculture, consumers indicated how much more they were willing to pay, out of 5 classes of price premiums; 3) demographics.	9.5% would not pay a premium for organic farmed seabass. High school degree or above and being the main shopper are more likely to pay a premium. Children in the household reduces probability of paying a premium. Gender does not affect value of the premium. > income, > premium.
Drake et al. 2006 [7]	North Carolina State University	Both	southern flounder	120 University staff and students	Sensory analysis of wild caught, saltwater farmed, and freshwater farmed. Fillets were poached in vacuum-sealed pouches. 8 trained panellists performed a descriptive analysis and developed the sensory lexicon. The untrained consumers evaluated overall acceptance, flavour, texture, appearance and freshness on a 9-point hedonic scale.	Saltwater farmed: sweet aroma; fresh fish flavour; sweet and salty tastes. Freshwater farmed: old fish and earthy flavour. Wild: old fish, sea complex, metallic, sour and medicinal flavours. Minimal texture differences. No differences in astringency or moisture release. ↑ consumer scores for saltwater farmed and wild on flavour, appearance liking and perceived freshness intensity. Saltwater farmed had the highest overall liking and freshwater farmed the lowest. Preference: saltwater farmed > wild > fresh farmed.
Verbeke et al. 2007 [8]	Flanders, Belgium	Both	General	429 mainly responsible for food purchasing. Age 19-83. Focus groups: 22 (6-8	Personal survey, at home or in shopping streets and supermarkets. 1) fish consumption habits. 2) awareness of nutrient and contaminant content in fish. 3) perception of farmed vs wild fish: 5-point interval scale; evaluate if wild is better than farmed in 6 attributes: health, safety, taste,	Most average perception scores are in the scale midpoint. 33% believe wild fish have a better taste; 28% believe that wild fish are healthier; 20% believe that wild fish are more nutritious. 33% believe that farmed fish are more easily available. 22% disagree that wild fish are safer.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				/ group). All female, age 25-57.	nutritional value, versatility and availability. 4) demographics. Focus groups: divided by light or heavy fish users.	Those >55 years hold a stronger belief that wild fish are healthier. Belief that wild fish have a better taste ↑ with age. Idea that wild fish taste better is stronger in families with children. Those with lowest income had a > favourable perception of farmed fish on safety and health. Gender, family size and education level not significant. Heavy fish consumers have a stronger belief that wild fish is safer. The issue of wild vs farmed was not spontaneously mentioned during the focus groups. Light fish users: not aware of the existence of farmed fish; after explanation, they associated wild fish as more natural, healthier, better in taste, more expensive, ''living free'' and ''happiness''; wild fish were perceived as being more resistant against ''everything''; parallels with intensive terrestrial livestock farming were easily drawn. Heavy users: more open to aquaculture, moderate views; belief that wild is fresher, but unsure this reflects on quality. low interest in fish origin.
Verbeke et al. 2007 [9]	Flanders, Belgium	Both	General	381 mothers aged 20-50.	Paper surveys distributed to mothers from primary and nursery schools to be filled at home. 1, fish consumption habits; 2, consumer attitudes toward fish; 3, perceived importance of sustainability; 4, subjective knowledge; 5, interest in information; 6, perceived consumer effectiveness; 7, preferences for wild and farmed fish.	Rejection of wild fish seems to be based on sustainability and ethical considerations, while refusing farmed fish is more because of a lower intrinsic quality expectations. There is a need for more communication to consumers about sustainability and ethical issues in relation to wild/farmed fish.
Li et al. 2008 [10]	North Central USA	FF	Hybrid striped bass	111 Supermarket, 36 chains and 208 restaurants.	Supermarket and chains: postal survey. Restaurants: phone survey. 1) preliminary: frequency of frozen fish purchasing; familiarity and preference for methods to freeze fish; frequency of fresh whole fish and fillets purchasing; ideal size of whole fish and fillets; familiarity with hybrid striped bass; maximum willingness to pay for whole and fillets of hybrid striped bass. 2) follow-up: source of info on fish products; preference for product forms (0-10 scale); factors affecting purchasing (0-10 scale of	General lack of familiarity with farmed hybrid striped bass. Only 25.8% of the money restaurants spent on finfish went to farmed fish purchases. Main sources of info: wholesalers, industry newsletters and trade publications. Respondents rated farmed fish high in areas that they thought to be important. For supermarket chains, perceived freshness of farmed fish was not at the desired level for this important factor. For some restaurant respondents, perceived quality and freshness of farmed fish were not on

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
					importance, 16 items); perception of farmed fish; expenses of each category; barriers to selling farmed hybrid striped bass.	par with their importance. Respondents claim their ratings are based mostly on perceptions. Customer familiarity and price are the main barriers to selling farmed hybrid striped bass.
Quagrainie et al. 2008 [11]	Indiana, USA	Both	General	870	Mail survey: seafood purchasing habits; willingness to pay for shell-on, headless freshwater shrimp, channel catfish fillets, hybrid striped bass fillets, tilapia fillets, yellow perch fillets from Indiana; demographics.	Factors that + affected interest in Indiana farmed products: previous purchase of farmed products; frequent at-home seafood consumption and age ≥36. Those who purchase wild seafood were not interested in Indiana farmed products. Those in the income group \$20,000–\$59,999 showed interest, households with > incomes were less interested. Consumers are WTP for Indiana farmed seafood products the range of market seafood prices. Consumers will not be interested in Indiana farmed products as premiums increase.
Gaviglio and Demartini 2009 [12]	Italy	Both	General	300 fish consumers	Personal surveys at fish shops, street markets and local markets. 1, sociodemographics; 2, product knowledge; 3, Perception of farm-raised and wild-caught fish; 4, important variables for fish purchasing.	50% always reads fish product labels. In 80% of the cases, knowledge appeared to be medium-high to high. 91% thought that wild-fish tastes better than farmed fish, but 57% admitted not being able to distinguish fish origin by taste alone. As to nutritional value, interviewees were evenly distributed between those who preferred wild fish and those who did not. Respondents generally believed farmed fish costs less than wild fish, and that it could help fish resource conservation efforts. They showed some reservations on the use of antibiotics in aquaculture. Almost half of the sample thought that it can pollute waters. Respondents stated that Italian (73%)) or European (69%) origin is an essential characteristic. 45% of respondents set price as “fundamental” or “very important”.70% were willing to replace a well-known but endangered wild species with a similar farmed product. The farmed averse group, which accounts for 33% of the whole sample, has two clusters, one representing hedonic and the other, old-generation consumers. The group disposed to buy farmed fish included three clusters: price-

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
						forced consumers, who prefer wild fish but can't afford it; trend-influenced consumers, who demonstrate attention to seller's opinion; and the market-attentive ones, with higher knowledge.
Honkanen and Olsen 2009 [13]	Spain	Both	General	450 responsible for food shopping	Personal survey with 7-point semantic-differential and 7-point Likert-related scales. Concern about fish welfare, environmental concern, ambivalence towards farmed fish, self-reported fish consumption, attitudes towards fish and farmed fish, health involvement, importance of food naturalness and information for food purchasing were assessed.	Farmed fish welfare issues do not seem important. Three segments were identified: the unconcerned (27%), the wild fish concerned (34.5%) and the ambivalent (38.5%). Attitudes toward farmed fish, the importance of natural food and social class were most important in profiling differences between clusters.
Kole et al. 2009 [14]	Netherlands	Both	Cod	1440, age over 12, not allergic to fish, no active dislike of fish.	150 g of fresh chilled cod fillet (Farmed or wild) were packed in modified atmosphere in a container with a label with the relevant information. The label also had a recommended consumption date, storage temperature and preparation instructions. Information: product method (none, farmed and advantages of farming, wild); freshness (eat before date or date since catch); RIVO quality seal or quality claim from retailer; price (3 levels). Then, consumers filled a paper survey regarding attractiveness, quality, freshness, healthiness, pleasantness, texture and taste of the product. Another survey was presented to tasters, about attitudes and perceptions in fish consumption	Overall appreciation of farmed cod was higher than wild if samples were unlabelled. Labelling a sample as farmed decreases its overall appreciation.
Altintzoglou et al. 2010 [15]	Belgium, Norway, Spain	Both	General	1319, randomly from the representative IPSOS European online access consumer panel. Equally distributed among 3 countries. 20-60 years.	Electronic survey. Questions about fish consumption; attitude toward eating fish. Then, participants were exposed to a specific message. 8 different conditions: safety; sustainability; health; health and safety; safety and health; safety, health, and sustainability; safety, health, sustainability and European dimension; no message. Participants were asked how they feel about the message. Safety, healthiness, sustainability, and convenience perception of farmed fish were measured.	Image of aquaculture fish is generally positive (safe, healthy, sustainable, and convenient food). This was not affected by any means of exposure to additional information. Consumers exposed to the information related to the EU origin and guarantees reported a more positive image of farmed fish. Belgian consumers reported that farmed fish is < safe, healthy, and convenient than other countries. Spanish consumers considered farmed fish production more sustainable than the other two countries.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				Responsible for food purchasing.		
Amberg and Hall 2010 [16]	U.S.	FF	Salmon	83 newspaper articles published in national and regional papers	Four online newspaper databases were searched using combinations of “farmed salmon” or “farm-raised salmon” with “health risks,” “contaminants,” or “PCBs”. Additional criteria: date range of July 28, 2003 - December 31, 2004; a discussion about contamination within the headline or the first few paragraphs; wire or feature only; and reference to either the EWG (2003) report or Hites et al. (2004) study. An examination of rhetorical markers of certainty and magnitude was performed.	Most statements presented info in a qualitative format, the least informative for readers. Most stories had a limited amount of high-precision info. Journalists’ rhetorical practices conveyed conflicting interpretations of the same data, leading to inconclusive messages about risks and benefits. Even highly precise numeric data were often presented in ways that were likely to confuse readers.
Ariji 2010 [17]	Japan	Both	Bluefin tuna	2400 respondents	Online survey. DCE, attributes: production method (full cycle farming, farmed post-caught, and wild), label (eco-label), origin and price. Questions were repeated after exposure to a message regarding overfishing and advantages of fish farming.	Before info was provided, the WTP for wild bluefin tuna was 77 yen higher than full-cycle farmed tuna and 49 yen higher than farmed tuna. WTP was 109 yen for sushi with an eco-label and 159 yen for the one labelled as domestic. After info was provided, domestic origin remained the most valued attribute, with an WTP increased to 229 yen. WTP for full-cycle farmed fish becomes greater than conventionally farmed fish.
Fernández-Polanco and Luna 2010 [18]	Spain	Both	Gilthead seabream	2394 consumers, randomly selected proportionally to the regional population, gender and age	Personal interviews. 325 seabream consumers, verified to be capable of distinguishing farmed and wild seabream, provided scores on sample quality of each. Survey: evaluation of quality, safety; perception of aquaculture. 1-5 hedonic scale or T/F.	Consumers consider that a seabream is safe if its physical quality is satisfactory and vice versa. High wild seabream overall quality assessment is linked with a low farm-raised physical quality score. Consumers perceive aquaculture as safe and sustainable. Appreciation of the wild species would improve the evaluation of the farm-raised alternative.
Myers et al. 2010 [19]	USA, northeast	Both	General	250 (50 per store, 5 stores).	In-store intercept survey with financial reward: social-demographics, live seafood consumption habits, information sources, attribute importance for purchase, perceptions of live seafood. Store traffic assessed.	Consumers value physical appearance of the product over price, generally have no preference for either “Product of the USA” or imported, and generally have no preference for either wild or farmed.
Olesen et al. 2010 [20]	Norway	FF	Salmon	115	In person choice experiment. Attributes: price; certification (organic, certified by KRAV vs Freedom Food, certified by RSPCA vs	The average consumer preferred organic and Freedom Food salmon to the conventional. They WTP a price premium of ≈2 €/kg (15%) for

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
					conventional); colour. Fillet colour was graded according to the Roche SalmoFan^{TM}. Survey: demographics, knowledge, opinions and attitudes. Overview: 1) explanation; 2), DCE, marking the answer on a card; 3) info about origin of colour; 4) more DCE (differing only in colour, no label provided); 5) info about production methods; 6) DCE; 7) each participant drew one card determining his or her binding scenario; 8) Each participant got the salmon fillet he or she had chosen in his or her binding scenario.	organic and Freedom Food salmon, compared with conventional salmon of the same colour. The combined effect of the organic label and the pale colour is significantly less than the utility of the conventional salmon. 54% totally agree that it is important that salmon feed is free from artificial pigments. Most agreed or partly agreed that organic food was healthier (56.5%) and that organic production was better for nature (74.6%).
Young et al. 2010 [21]	UK	Both	Tilapia	Focus groups: 90, broad spread among the standard socioeconomic criteria of age, gender, socioeconomic class and education levels. Farmers: 17	Observational information, consumer focus groups, product placement and in-depth interviews. Focus groups: explored participants' attitudes towards health, food and fish; perceived health benefits of fish, sustainable food production, organic fish and awareness, perceptions and purchase habits concerning tilapia, fish quality, freshness, packaging. Product placement: gastro-pub and Michelin-star restaurant. Semi-structured interviews held with buyers in restaurants, retailers, fish wholesalers and processors. In-depth interviews: farmers, focus on diversification concept.	Focus groups: strong interest in environmental issues. Some WTP for environmental attributes, but perceived freshness, quality and size will be essential. 3 target groups: ethnic consumers, green consumers and discrete segments (gastro-pubs and upscale fish restaurants) within food service. Product placement: very positive reviews. Except for one TV 'celebrity chef' who won't serve farmed fish on their restaurant. Chefs reported a WTP 'reasonable' premiums if quality and other attributes were maintained. Farmers: the perceived risks outweighed the potential returns for most to develop a pilot system.
Altintzoglou et al. 2011 [22]	Belgium, Norway, Spain	Both	General	1319, randomly from the representative IPSOS European online access consumer panel. Equally distributed among 3 countries. 20-60 years. Responsible for food purchasing.	Electronic survey. 1, Questions about fish, farmed fish and wild fish consumption; 2, attitude toward eating fish, measured on seven-point semantic differentials for four bipolar items; 3, involvement in health, measured using the six statements; 4, socio-demographics.	Positive correlation between involvement in health issues and fish consumption, for both farmed and wild fish. Consumers from Belgium displayed a higher farmed-to-wild fish consumption ratio than Norway and Spain. Consumers in all countries were poorly aware of the origin of the fish they consume.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
Hansen and Onozaka 2011 [23]	Norway	FF	Salmon and tilapia	Exp 1: 194 university students Exp 2: 216 consumers (market research database)	Exp 1: in the form of a newspaper article. One group was informed of a new salmon product from Chile where the industry had suffered from the salmon disease, but the importer guarantees the product to be safe. The second group received the same information, except it was tilapia. 2nd variation: Canada was the source unaffected by the outbreak. fish was either from Chile or Canada, creating a total of 4 experimental cells (salmon/tilapia × Chile/Canada). Purchase intentions measured (7-point Likert scales). Exp 2: similar, but fish from either Canada or Chile purchased from either a known upscale retailer or a discount retailer (evaluate brand association).	Species/country directly affected (Chilean salmon) had the lowest purchase intentions and perceived quality. Significant difference in perceived quality between Canadian and Chilean tilapia and Canadian tilapia and Canadian salmon. Significant negative spillover effects on the same species produced in unaffected countries and on other species farmed within the affected country. No significant differences in scores between the two supermarket chains, suggesting no significant mitigating effects by associating with a retailer with a good reputation.
Pohar 2011 [24]	Slovenia	Both	Brown trout	34, average connoisseurs of fish gastronomy with consumption between once a week and once a month	Fish of approximately the same size among wild and farmed were purchased and frozen. For analysis, fish were thawed, sliced, salted (2%), floured and pan-fried.	75% of consumers stated that they noticed a difference between wild and farmed specimens. For appearance and aroma, wild trout was preferred; for juiciness and mouth feeling farmed trout was favoured. Overall, 52% of the panel preferred farmed trout.
Rudd et al. 2011 [25]	Canada	Both	Salmon	799, consumed salmon at home in the past year and responsible for food purchasing.	Online DCE. Questions about risk perceptions, familiarity with salmon products, perceptions of farmed vs wild salmon, and demographics were also included. Attributes: global environmental impact (low, moderated or high), local environmental impact, level of omega-3 fatty acids (high or low), contamination (high or low level of PCBs, polychlorinated biphenyls), origin (Chile and Newfoundland), price.	Most agree that farmed fish is more available than wild. 30% disagree, while 54% neither agree nor disagree, that farmed fish has a better taste. 38% agree that farmed fish is safer (23% disagree). Perceptions of health and nutrition are varied. Contaminants and antibiotic residuals were clearly viewed as most important environmental threats of fish farming. Decreased contaminants and farmed in Newfoundland led to the highest WTP values.
Solgaard and Yang 2011 [26]	Denmark	Both	Rainbow trout	1000. Age 18-65. responsible (or partly) for the household's food shopping	Online survey. 1) introduction with 5 criteria that must be met to guarantee good fish welfare; 2) general questions about fish welfare and farmed/wild fish consumption habits; 3) willingness to pay for trout with a label ensuring it was raised according to good fish welfare criteria	37% believe fish have better welfare in Denmark than other EU countries; 48% do not believe so. 62% do not believe fish have better welfare at larger farms than at smaller farms. 64% believe fish have better welfare now than 20 years ago. 80% believe wild fish have better welfare than

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					(Y/N with open-ended question about value); 4) demographics.	farmed. 75% very much like or like farmed fish; 92% indicate they like or very much like wild. Main reasons for disliking farmed fish: taste and concern over medicine residues; for wild: taste and smell. 45% buy farmed fish ≥1x/month. 51% buy fresh and 42% buy frozen wild fish ≥1x/month. 52% were not WTP extra for welfare trout. 48% were on average WTP 25% extra for welfare trout. Women, those with a longer education, in higher income households, older, who emphasize eco-friendly production of welfare fish, freshness, and animal welfare are WTP extra.
Vanhonacker et al. 2011 [27]	Belgium, Norway and Spain	Both	Salmon, cod, tuna, trout, seabass, seabream, Alaska pollock, mussels, shrimps, mackerel, saithe, herring, redfish, hake.	1319, responsible for food purchasing, balanced age distribution within the range of 20-60 years, randomly selected from the representative IPSOS European consumer access panel.	Online survey. 1) consumption of fish, farmed and wild; 2) demographics; 3) involvement with fish and subjective knowledge; 4) Perception of farmed fish, measured 24 bipolar items (3 items for each health, safety, quality, convenience, sustainability, animal welfare, price and sensory), using a 7-point semantic differential with the negative item on the left side of the scale and its antonym (positive) on the right side; 5) consumer attitudes (interest in fish origin) and objective knowledge (T/F statements and how certain they were about the answer on a 7-point Likert scale); 6) list of species provided, ranking of consumption frequency.	Perception of farmed fish was mostly neutral. Limited awareness of fish origin. + association of farmed fish with a sustainable alternative to wild fish. Convenience issues were scored relatively high. Health, safety and sensory issues appeared in the top of the list. At the bottom, issues dealing with animal welfare were found. Between country-differences were rather minor. Interest in fish origin ranked 9th out of 14 cues (most preferred were quality, health and safety). Low overall objective knowledge and high uncertainty. 3 groups. 1) predominantly wild fish consumers: highest involvement and subjective knowledge. higher total fish consumption frequency. perceived farmed fish more negatively in terms of safety. less convinced about the better price and convenience of farmed fish. In Belgium and Spain, claimed consumption of predominantly wild fish was not well reflected into preferred fish species. Most interested in fish origin. 2) predominantly farmed fish consumers: more females and < age. lowest fish consumption. High share of Belgians. lowest interest in fish origin. reported choice for farmed fish was based on (perceived) food characteristics. 3) mixed group.

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Whitmarsh and Palmieri 2011 [28]	Scotland	FF	Salmon	745	Postal survey. 6 indicators, 3 on aquaculture socio-economic benefits (employment and livelihoods; edible supplies of fish; and tax revenue) and 3 on environmental damage (pollution and water quality; visual intrusion; impacts on wild stocks). Analytic hierarchy process used to measure relative importance.	Respondents were more likely to buy salmon on a regular basis if age > 30, employed in managerial or professional posts and resident in the mainland regions. The higher the priority attached to minimizing the environmental impact of salmon farming, the lower the likelihood that salmon would be purchased on a regular basis.
Claret et al. 2012 [29]	Spain	Both	General	Focus groups: 81;, involved in food purchasing. Conjoint analysis: 914..	9 Focus groups from different Spain regions (each 6-12). Conjoint analysis: survey. 9 different fish profiles were established and consumers ranked from 1 (first purchase option) to 9 (last option). Attributes: origin; production method; product form; price.	Focus groups: most relevant factors for choosing fish were those that appear on the label by law. Similar in all regions. Conjoint analysis: most relevant factor COO (own country preferred) > storage (fresh preferred) > price (6 & 12€/kg were acceptable, but not 18) > method (wild preferred). Segmentation: 1) 26.7%; all attributes equally important when buying fish; 2) 19.6%; COO most important; farmed fish preferred; 3) 12.4%; COO most important; remaining factors are similar; 4) 16.3%; interest in all attributes but storage; 5) 25%; price most important.
Davidson et al. 2012 [30]	Hawaii, USA	Both	Salmon, tilapia, tuna, moi	566 respondents.	Mixed survey (online and in-person). 1) attitudes and perceptions towards aquaculture; seafood consumption habits; demographics 2) DCE. Attributes: production method; form; origin; price.	65% eat seafood a home weekly. Main species: shrimp, tuna, salmon. Reasons for seafood consumption: taste, health. 63% noticed labels specifying if seafood is farmed or wild. 60.8% ranked label influence as important for purchase. Wild seafood is favoured primarily for taste preferences. DCE: more likely to choose the product if it is wild. Less likely to purchase higher priced fish. Less likely to purchase frozen products. WTP for wild > farmed (in salmon, \$5.02/lb more for wild; \$10.94/lb for tuna; \$2.09/lb for moi). Utility for natural vegetable-based feed in tilapia production was not significant. Wild or marine farmed moi are preferred to land-based aquaculture.
de Moura et al. 2012 [31]	Portugal	Both	General	8 women; responsible for purchasing and preparation of fish	Two focus groups divided by education level. Benefits and barriers related to fish consumption and fish choice criteria; general perceptions of farmed vs wild fish; farmed vs wild fish risk perceptions.	Reasons for consumption: health, taste. Difficulties: family preferences, inconvenience. Higher educated women had difficulty judging fish freshness and did not know how to cook it. Not all participants are aware of the existence of farmed

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						fish in the market. Lower educated women claim there is a difference in taste and that farmed fish is cheaper. Farmed fish are perceived as being safer than farmed meat. Pollution is perceived as a bigger risk in wild-caught.
Freeman et al. 2012 [32]	Germany & Israel	Both	General	431 in Israel, unknown in Germany	Written face-to-face interviews. Categories: environment, tourism, government and policy, employment and job security, health and lifestyle, political and community participation, aquaculture, and demographics.	Public perceives coastal tourism and mariculture as legitimate uses of the coastal zone that need not be in conflict. Higher familiarity with aquaculture in Israel than Germany. For Israelis: major environmental concern is fish-farm effluent and pollution of marine environments; negative correlation between environmental concerns and support for aquaculture. In Germany: positive relationship between environmental concerns and attitudes towards mariculture may relate to concerns about wild fish stocks depletion.
Lawley et al. 2012 [33]	Australia	Both	Barramundi	145, main grocery buyers, who purchased chilled or fresh fish for consumption in-home over once per month, and consumed barramundi over three times a year. 26 for focus group.	Quantitative sensory evaluation of 9 cooked fish species. Fish lightly pan fried with minimal oil and no seasoning. Evaluated: overall liking, flavour, aftertaste, mouthfeel, sweetness, oily flavour, bitterness, saltiness, colour, aroma. Qualitative focus group: 1) focus on the prior product experience in the sensory evaluation, intrinsic product attributes and reasons for acceptance /rejection. 2) what extrinsic variables were important in consumers' quality evaluations, and how these extrinsic cues combined with intrinsic cues to arrive at over-all quality evaluations.	Sensory analysis: Goldband snapper with highest overall liking; tank barramundi with lowest. Wild barramundi < sea-cage and earth-pond barramundi. Earth-pond with highest flavour strength. Liking of taste and aftertaste (0.82) were the most highly correlated with overall liking. Focus groups: most consumers still lack knowledge and confidence when buying, preparing and cooking fish at home. COO important for purchasing. Consumers prefer labels to be "simple". There was little knowledge of farmed versus wild.
Roheim et al. 2012 [34]	Rhode Island, USA	Both	Salmon	250 seafood purchasers	In-person interviews at supermarkets, seafood markets, farmer's markets. DCE attributes: price; production method; certification (meets standards vs none); certifying body (government agency, environment organization, no info). Definition of standards provided, 'only approved antibiotics are used', 'water quality clean', fish not 'densely stocked', 'feed sustainable and approved'. Survey with seafood attitudes and preferences, beliefs on the quality of farmed products, perception of	50% agree or strongly agree that fresh farmed fish tastes better than frozen wild; 53% believe farmed fish' feed is not natural; 42% agree that farmers control water quality; 43% disagree or strongly disagree that farmed fish is fresher than wild; 50% agree that farmed fish may have been treated with antibiotics (31% unsure); 42% unsure about government regulation of fish farming; 32% unsure about environmental impacts of aquaculture. Simply stating that the product

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					farming practices, important factors for seafood quality.	meets standards does not affect probability of choice. Indicating certification by a governmental agency or by an environmental organization ↑ the probability of choice. Probability of purchase ↓ as price ↑. Farming ↓ purchase probability. Purchasing at seafood market less likely to buy certified salmon. Lacking knowledge about farming practices and women > likely to buy farmed salmon. Those who believe aquaculture has an environmental impact are < likely to buy farmed salmon. Certification by a governmental agency is > valued than by an environmental organization. Wild products are preferred even when farmed are certified.
Stefani et al. 2012 [35]	Italy	FF	Seabream	251, fish and seabream consumers; responsible for food purchasing. nationally representative	In-person paper survey. 1) fish and seabream purchasing habits; 2) DCE attributes: price; origin; farming method (organic or conventional); place of farming (marine cages or ponds); feed (fishmeal and vegetable meal or only fishmeal); 3) follow-up questions: would the consumer change purchase frequency if the chosen product was the only one available on the market; if so, how many annual purchases would be made; 4) attitudes about fish consumption and the environment; 5) demographic data.	Most agreed on the safety of farmed seabream, its benefits for health, and the ease of access on the market. Italian origin had the strongest effect, followed by rearing in marine cages, with organic production in 3rd. Higher attitudinal scores toward the environment related to organic aquaculture and use of marine cage. Feed variable not significant. Low importance to price was observed for those with higher scores for ‘‘health’’. Those with a ‘‘critical’’ attitude toward the environment attributed greater importance to the organic production. Highest marginal WTP is Italian origin (median €18.1), followed by production in marine cages (median €6.75) and organic (median €2.76).
Cardoso et al. 2013 [36]	Portugal	Both	General	1083.	Online survey. 1) consumption preferences (production, processing); 2) consumption frequency (species); 3) consumed quantity per meal (species); 4) culinary treatment; 5) demographics.	62.9% prefer wild to farmed fish, but only 3.2% refuse farmed fish. Preference for fresh, whole fish. Main species/products: soaked cod, hake, canned tuna, gilthead seabream, salmon. Men prefer wild fish to a higher degree (vs women). People from inland areas are less attracted to wild fish and eat more seabream and seabass.
Fernández-Polanco et al. 2013 [37]	Cantabria, Spain	Both	Gilthead seabream	169 consumers (sample representative	DCE. Attributes: harvest method; origin; sustainability claim; safety claim; health benefits; price (4 levels). Survey at a food market.	All attributes impact fish purchasing in the consumer segment. Relative importance: price > origin > harvest method > sustainability > health >

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				of consumers typically purchasing fish at traditional markets) and 27 retailers.	Definition of 'sustainable fisheries' provided. 8 DCE tasks, then attitude questions and demographics. Retailers were asked to choose between the different alternatives, imagining they would make choices representative for their consumers and considering the price as a recommended consumer price.	safety. Wild preferred, WTP of €6.42/kg. WTP for domestic (vs imported) is €6.84/kg. In retailers' perception, consumers are more price-sensitive and less influenced by product claims than consumers reveal.
Grimsrud et al. 2013 [38]	Norway	FF	Salmon	771, a representative (based on age, gender, region and education level) panel of Norwegian households was selected from TNS Gallup's pool of 60,000 Norwegian citizens of 18 years and above.	Online survey. 1) knowledge and attitudes towards fish welfare and farming; 2) DCE; 2 breeding programs and a standard breeding – no improvement of welfare but no extra cost. Attributes (Y/N): ↓ deformities; ↓ aggressive fish; ↑ resistance to salmon lice; ↑ resistance to infections; price (presented as an extra earmarked tax, NOK per household per year). 3) demographics. Respondents were informed that salmon quality in stores would remain since all abnormal/sick fish are discarded & that spending more money on breeding programs to improve fish welfare would reduce the money available for other purposes. The four attributes were first described using text and photos.	45% of households purchase farmed salmon 1–2x/month. 41.2% has avoided purchasing seafood because of animal welfare concerns, and of these 26.4% has avoided purchasing farmed fish. Women derive a ↑ utility from breeding programs that focus on reducing deformities, improving salmons' resistance to disease and lice. Older, reside in cities or suburbs, or have higher incomes are WTP a ↑ earmarked tax. Average WTP is higher for lice.
Hall and Amberg 2013 [39]	Idaho and Washington, USA	Both	General	1159 seafood consumers	Mail survey. 1) personal seafood preference: importance of taste, familiarity, price, and freshness. Assessed on a 7- point scale. 2) beliefs related to seafood in general and aquaculture in particular: perceived health value of seafood; relative quality of farmed vs wild seafood, environmental and economic benefits of aquaculture, environmental and health problems from aquaculture, and contaminant concerns. Same 7-point response scale, with a ''don't know''. 3) awareness of media attention to aquaculture; 4) seafood consumption; At the beginning, aquaculture was defined.	Respondents agreed that seafood is healthy and they preferred wild over farmed products. Many were uncertain about health and environmental benefits and problems associated with aquaculture. They agreed that aquaculture reduces pressure on wild fish, but also that it has the same problems as other agricultural practices. Belief in the superiority of wild seafood was a strong predictor of consumption choices. Belief in the benefits of aquaculture was + related to higher consumption of farmed products, but beliefs related to environmental and health problems associated with aquaculture did not predict specific consumption. ≈50% recalled hearing or reading about aquaculture in the mass media and recall of negative stories contributed to a general preference for wild products.

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Kalshoven and Meijboom 2013 [40]	Netherlands	Both	General	8 buyers belonging to the chain between fishers/farmers and consumers	Semi-structured personal interviews with open-ended questions about their personal experience in the matter, similarities or differences between wild sea and farmed fish, dilemmas in their daily work as a fish buyer and the future of their sector.	Moral values are used in their work, but also result in a number of value conflicts. The focus on sustainability and animal welfare seems driven by external and market factors. Fish welfare is seen as part of sustainability. Welfare seems more important for farmed than for wild fish as the buyers feel responsibility regarding these kept animals. The decision of if a product is sustainable is mainly based on labels, but authors claim a low relevance of these labels for addressing the ethical dilemmas of buyers.
Kupsala et al. 2013 [41]	Finland	FF	Salmon	1890, representative of population.	Postal survey. 1) demographics. 2) perceived welfare of several farmed animals (5-point Likert scale); perceived welfare of farmed fish; evaluation of statements regarding animal mental ability.	Only 13% regard salmon welfare as fairly poor or very poor and it was better than the welfare of pigs and poultry. Salmon are rated low in their mental abilities, including the capacity to feel pain. Results imply that there is no urgent consumer pressure to improve the conditions of farmed fish in Finland.
Mauracher et al. 2013 [42]	Veneto, Italy	FF	European seabass	366, no younger than 30, chosen to reflect the real percentage composition of the resident population	Face-to-face interviews, in supermarkets and fish markets. If the consumer was unfamiliar with organic fish, the interviewer informed that organic production implies water quality control, respect for the environment, attention to animal welfare, ban on chemicals and GMO and that all organic productions are controlled by independent institutions, authorized by the Ministry of Agriculture. DCE attributes: origin, size, production (traditional farming vs organic); price. Survey: 1) frequency of seabass consumption and reasons for non-consumption; 2) fish consumption habits; 3) knowledge and consumption of organic food; 4) demographics	50% purchases organic products. Reasons: higher quality, no GMOs. Two clusters: organic potential consumers and conventional. Both prefer Italy-produced. Overall higher WTP for the COO than for the breeding method used. Organic potential consumers prefer bigger size, conventional prefer smaller. Conventional are indifferent to organic production.
Pieniak et al. 2013 [43]	Czech Republic, Germany, Greece, Italy, Portugal, Romania, Sweden and UK	Both	General	3213, ~400 in each country. Age, 18–70 years. Responsible for food purchasing within the	Online survey. 1) fish consumption; 2) objective knowledge about fish and aquaculture was measured with 6 T/F statements; 3) interest in info fish included 9 information cues (3 mandatory and 6 potential); 4) information sources. All assessed with 5-point Likert scales.	Highest consumption frequency was in Portugal. 70% of the Portuguese participants reported eating fish >1x/week. Objective knowledge was low. Southern European consumers had the most correct answers. Most interest in a quality and/or a food safety label. Least interest in info about FAO fishing zones. A strong interest in nutritional

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				household. Representative within each country for age and region.		info was noted in Greece, UK, Romania, Portugal and the Czech Republic. Participants from Germany, Italy and Greece were interested in production method (wild/farmed). The most frequently used info sources were labels and sellers in retail outlets or supermarkets, followed by the Internet and television.
Rodríguez et al. 2013 [44]	Spain	Both	Seabream	Data obtained from FAO datasets (1985-2009).	Johansen methodology: cointegration of the price series of farmed and wild gilthead sea bream was tested.	Farmed seabream price was higher in early production stages, and decreased over time as production grew, until stabilization in 2002. Wild had initially low prices (local product, poorly known and consumed outside these markets), then price grew (associated with an aquaculture crisis), until stabilization at a higher price than farmed. Wild and farmed seabream form two heterogeneous products in the Spanish market.
Schlag and Ystgaard 2013 [45]	France, Italy, Germany, Greece, Norway, Spain and the UK	Both	General	208, male /female ratio of 40/60, consumers of fish. In each country, there was a segmented convenience sample of young professionals, mature professionals, housewives/husbands with children and socio-economic strata C/D/E	Focus groups.	Aquaculture is seen as profitable with economic benefits for the locals. Participants say it may affect traditional livelihoods and long-established fishing, particularly in Spain where fish farming is associated with big industries run by a few rich conglomerations. >20% say that wild fish is expensive and farmed is cheaper. Habitat destruction and biodiversity reduction are associated with farming; overfishing and ocean pollution to traditional fishing. This is heightened by participants' lack of knowledge and the perception of high scientific uncertainty. Spanish, British and French are less aware of environmental topics than other Europeans. No distinction between farmed and wild on health impacts. Respondents express distrust towards fish farming. A particular concern is the "unnaturalness" of farmed products.
Vanhonacker et al. 2013 [46]	Czech Republic, Germany, Greece, Italy,	Both	Seabass and seabream	3213, responsible for food purchasing and fish	Online survey. 1) Fish (generic, wild, farmed, seabass, seabream) consumption frequency. 2) image of generic fish (1, very good to 4, poor image + no opinion). 3) image of farmed, wild,	Wild fish had a > positive image than farmed fish. Farmed fish was evaluated = to generic fish in Romania, Sweden and Czech Republic, slightly < positive in Germany, Italy, Greece and Portugal or

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	Portugal, Romania, Sweden, UK			consumers, selected from the Ipsos Online Access Panel. Quota control variables were rural versus urban environment and age, in the range 18–70. 65/35 female–male ratio, represented the selection of the person mainly responsible for food purchasing within the household.	seabass and seabream, with 5-point scales (1, positive verbatim; 5, negative verbatim): good vs poor for health; respectful vs not respectful for the animal's welfare; good vs bad quality /price relationship; fresh vs not fresh; respectful vs not respectful for the environment; quality guaranteed vs not guaranteed; widely vs seldom available.	< positive in the UK. Very healthy image of fish. Fish products were perceived as readily available. Neutral opinions for the price–quality ratio and the quality guarantee. Farmed fish were mainly favoured in availability and price–quality ratio, and wild in health. Image of farmed and wild fish matched well with the generic perception of fish. In Germany, farmed was associated with > freshness than wild. Greece favoured wild.
Claret et al. 2014 [47]	Spain	Both	General	Focus group: 81, involved in food purchasing. Survey: 919, involved in food purchasing and preparation at home, with quotas including gender (minimum 25% men) and age (between 20 and 75 years) within each region (according to the distribution	1) 9 Focus groups in different Spain regions – assessing consumer perception about wild and farmed fish. 6-12 people each. Generic discussion on fish consumption, motives / advantages and barriers / disadvantages of fish intake, and perceived differences between farmed and wild fish. 2) Survey. Demographics; objective (5 T/F statements) and subjective knowledge (7-point Likert scale) about fish; 19 items about consumer beliefs on farmed and wild fish (safety, quality, control, availability, price).	Focus group: similar results in all regions. A lack of knowledge of ever having tasted farmed fish was seen, but they claimed to consume species that are mainly farmed. Participants stated that they perceived tangible differences between both kinds of fish. Survey: no significant differences on safety for the two production methods. Farmed fish perceived as less affected by pollution, heavy metals and parasites. Wild fish perceived as having higher quality. belief concerning artificiality was the strongest when comparing farmed versus wild fish. Moderate-high level of both objective and subjective knowledge. 3 clusters: "Traditional/conservative", "Connoisseur" and "Open to aquaculture".

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				of the Spanish population).		
Guy and Nottingham 2014 [48]	Australia	Both	Mulloway	73 fish consumers	Fish from commercial farms or catches. For consumer assessment, wild mulloway, farmed mulloway and farmed barramundi were considered. Fillets were cooked to an internal core temperature of 70 °C. Tasting order was randomized, and samples were coded. Consumers were briefed on the tasting techniques (sniffing and mastication). Questionnaire: 5 unstructured line scales with endpoint anchors, required to complete it in a set sequence from aroma, colour, flavour, and texture to overall liking. Consumers were invited to make comments.	Farmed mulloway had > fat and energy than wild mulloway and were an excellent source of long-chain omega-3s with good fillet yields. Wild mulloway had > sodium but were a poor source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Consumer acceptance showed a strong preference for farmed barramundi over farmed mulloway in aroma, flavour, and overall liking, while wild mulloway and farmed mulloway differed significantly only in flavour.
Chen et al. 2015 [49]	France	Both	Salmon, Cod, monkfish, pangasius	116 fresh fish consumers, who participated in previous fish experiments, + 78 who were new	Choice experiment. Attributes: species, wild or farmed, eco label (MSC, none, AB label [French organic label]), origin, price range, into 8 possible combinations. To reduce the hypothetical nature of the experiment, real fish loins (300 g package) were used.	Premium for MSC-labelled wild cod was 4%. Premium for Agriculture-Biologique-labelled farmed salmon and farmed cod was 11%. When participants receive negative environmental info on farmed or wild fish WTP falls by more than the positive effect of ecolabeling.
Ellingsen et al. 2015 [50]	Norway	FF	Atlantic salmon	2147 respondents: representative (in gender, education and geographical location) panel of Norwegian households was selected from TNS Gallup's pool of 60,000 Norwegian citizens	Online survey: 1) attitudes to fish welfare and fish farming; 2) respondents willingness to pay for salmon improved welfare; 3) Demographics.	Animal welfare was not seen as a priority for government money application. On a 6-point scale (completely disagree to completely agree), mean of 4.5 on "farmed fishes right not to suffer equally important as humans". 63.7% know "little" about fish farming. Mean WTP for welfare assured: 51.7 NOK/kg.

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Feucht and Zander 2015 [51]	Germany (Stuttgart, Leipzig and Hamburg)	FF	General	6 groups of 7-12 consumers (total 56). Quota sampling was used with respect to age and employment. 50% aged 20-45, 50% aged 46-70. 33-80% worked full or part-time. No employment in agriculture, fisheries, food industry and market research.	Focus groups. 3 groups of consumers of conventional food; 3 groups also consume organic food. Participants were asked about their knowledge of sustainable aquaculture. Then, they were informed about production methods (earth pond, flow-through system and RAS). They discussed their attitudes towards these systems. Next, participants were exposed to the messages: • Near-natural production; • Plenty of room to move; • Minimal drug usage; • No environmental pollution through nutrient run-off; • Minimal usage of fishmeal and fish oil in order to protect the oceans. Discussed the purchase relevance of these. The main sustainability labels (3 regarding organic, WWF and ASC) were also presented and discussed.	Sustainable aquaculture seen as a natural way of production that respects the environment. Fish feed was expected to be sustainable and species appropriate. Some judged the use of FM and FO as unsustainable and associated it with cannibalism. Several feared that feeding animal products to fish might promote illnesses. A farmed fish's life should be stress and pain free. Fish welfare perceived as a quality indicator. Most expected sustainable aquaculture to restrain from drug usage. Only a few approved medication to treat sick fish. Organic fish farming perceived as the ideal aquaculture practice, not distinguished from sustainable. Participants prefer European and doubt the quality of Asian products. Many were familiar with earth ponds and flow-through systems but RAS were mostly unknown. Visual appearance and description of earth ponds were closest to the expectations of a sustainable aquaculture. Lower stocking rates in earth ponds and flow-through systems compared to RAS appreciated. Many perceived RAS as the most industrialized and unnatural method. Participants were dissatisfied with all messages presented, too imprecise and too difficult to comprehend. Most did not recognize any of the presented labels from fish products.
Koral et al. 2015 [52]	Turkey	Both	Horse mackerel	8 trained panellists	Wild and farmed specimens were gutted, cleaned, brined and drained and then smoked. Along with analysis of proximate composition, oxidation, microbiological counts, the appearance, odour, taste and texture of fish were assessed by the panellists, using a 1-10 scale for each attribute.	Farmed specimens had a better appearance, texture, odour, tase and overall liking than wild during the whole study.
Polymeros et al. 2015 [53]	Crete, Greece	Both	General	149, responsible for purchasing decisions.	Personal interviews. 1) fish consumption frequency, place of purchase, product form, price, origin; 2) demographics.	Two clusters. 1) 67% of sample. Most with secondary education and ↑ age. ↓ income. ↓ preference for farmed fish. ↑ fish consumption frequency. Prefer fresh and raw fish. Low potential aquaculture consumers. 2) 33% of sample. ↑ education, ↓ age. ↑ income. ↓ fish

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
						consumption frequency. ↑ preference for farmed fish. Prefer frozen and processed fish. High potential aquaculture consumers
Alexander et al. 2016 [54]	Ireland, Israel, Italy, Norway and UK	FF	General	Age restriction 18-64. 2520. Evenly split according to gender and age.	Internet survey. (i) understanding of aquaculture benefits and impacts and IMTA; (ii) DCE; (iii) Perceptions of fish products and buying behaviour and (iv) demographics. During the survey, participants were shown a diagram of an IMTA system (after being asked if they had heard of the term), along with an explanation.	Main benefits: health and nutrition; reliable and affordable food source; prevents overfishing; economic benefits. Main negative impact was pollution. Mediterranean countries valued job creation, overfishing prevention, reliable and affordable food source and improved health and nutrition. Age >45 → < ranking high benefits. Males → < seeing aquaculture as job creation or improved health and nutrition. < education → < seeing aquaculture as a reliable and affordable source of food or as preventing overfishing. Prior awareness about IMTA was low. After explanation, improving overall sustainability, increasing food production and improving waste management were acknowledged by 70% of respondents.
Alexander et al. 2016 [55]	Cyprus, Ireland, Israel, Italy, Norway, Scotland	FF	General	48 stakeholders: community org; environmental; fishers; industry rep; planners; processors; producers; regulators; research; retail; suppliers; tourism	In-depth interviews, carried out with a topic guide. The guide was split into: 1) awareness and understanding regarding IMTA – if there was no awareness the interviewer would explain or show an explanatory video; 2) perceptions of IMTA; 3) risks to development of the industry and how to mitigate it; 4) industry image and impacts of IMTA upon it.	Those directly linked to aquaculture are more familiar with IMTA. In Norway and Scotland, stakeholders were > aware of IMTA. Raised concerns: spatial issues, food safety and disease. Perceived benefits: waste utilisation, lower impacts to the benthos, filtering of sea lice, creation of new income streams. Overall, IMTA was seen as able to mitigate the perceived current negative impacts of aquaculture. Stakeholders believed that IMTA could contribute to improving the image of aquaculture. Many stakeholders in Cyprus appear to generally be against aquaculture, suggesting that it cannot cover the needs of the growing population and that it conflicts with fisheries.
Ankamah-Yeboah et al. 2016 [56]	Denmark	FF	Salmon	18,471 observations from 2342 households (consumer	For each shopping trip, household reports food purchases: date and time, shop name, expenditure and. For each unit of salmon, households report info on organic label; a brand or private label; fresh or frozen; smoked,	There is a price premium of 19.7% for organic farmed salmon in retail markets in Denmark.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				scanner data). Refreshed panel data (20% of household replaced each year by a similar type).	marinated, breaded, stuffed or processed in another form; filleted or whole-fish; and, purchased on a special offer or at the normal price.	
Banovic et al. 2016 [57]	France, Germany, Italy, Spain, UK	FF	General	60. Consumers and purchasers of aquaculture products; have cooking and evaluation skills regarding fish; interested in new fish products.	2 focus groups per country (6 participants each group). 1) perception towards new food products ideas; 2) perceptions towards and modification of new aquaculture product ideas: defining product personality (personification association), defining the product (word association), modifying existing product ideas (role playing); 3) creation of new aquaculture products: i-profiling the typical 'user' of new aquaculture products (creative techniques, empathy map with brainstorming), ii-involve consumers in the process of product ideas creation (creative technique, storyboarding with brainstorming), iii- voting for the best product ideas (choice technique).	Fish described as a flexible, self-persevering, possessing camouflage ability, sociable and actively supporting and promoting environment and healthy lifestyle. Modifying existing product ideas - changing packaging, improvement of labels (more information on ingredients, product traceability and production) Profiling the consumer - typical consumer of new aquaculture products as a 'single-living', 'busy-lifestyle working person'; main goal of this fictitious consumer would be to pursue health and well-being. New product ideas – Emphasis on transparent and see-through packaging, convenience in preparation and additional preparation suggestions.
Bronnmann and Asche 2016 [58]	Germany	Both	General	Data from the German scanner household panel dataset	Attributes: species; product form; process form; brand; package size; price; production.	Premium of cod, tuna and plaice relative to salmon. Fish products from aquaculture have a higher value than wild fish.
Bronnmann et al. 2016 [59]	Germany	Both	Salmon	Data taken from a German homescan panel dataset on food purchases of households covering the period 2006-2010	Daily, households scan their food purchases, the point and date of purchase, several product characteristics (e.g., brand), as well as the European Article Number Code at home, using a handheld scanner. The dataset includes sociodemographic information. The QUAIDS model was used for specification of the fish demand system to derive income as well as own-price and cross-price elasticities.	Farmed and wild seafood are differentiated products with a good level of interchangeability in demand. Farmed salmon prices are higher than wild. This may be due to domestic stock shortages and the rising demand for fish in Germany, leading suppliers to import larger quantities of farmed fish. The additional transport costs may have increased prices. No differences between the elasticities of farmed and wild salmon, although farmed salmon is more expensive.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
Claret et al. 2016 [60]	Spain (Catalonia, Basque Country, Madrid)	Both	Black spot seabream, gilthead seabream, seabass, turbot	2 groups of 300 (informed and blind) that consume fish over twice a week.	Sensory analysis: fish fillets were cooked at 110 °C. All samples had been previously frozen for up to a month. Participants evaluated 8 samples (4 species × 2 production methods). One group received samples coded, the other received samples identified with species and production method. Overall liking: 10 cm semi-structured scale anchored in the centre (0 extremely dislike; 10 extremely like). Participants also filled a survey: demographics; fish consumption habits; beliefs about farmed fish (safety, quality, control, availability, price) – 7 point Likert scale.	Informed condition: 55.6% prefer wild fish, 26.6% have no clear preference. Blind: 39.7% prefer farmed fish, 40% have no clear preference. Sociodemographics, fish consumption and beliefs about farmed vs wild did not differ in the blind condition. Informed condition: consumers preferring wild fish were those who perceived wild fish as being healthier, having a healthier diet, having better taste and being of better quality; consumers preferring farmed fish considered these to be less affected by marine pollution and to provide more guarantees than wild fish.
Darko et al. 2016 [61]	Tanzania (Morogoro, Mbeya, and Mwanza)	Both	Tilapia	479 households.	DCE survey. Attributes: price, production method; size; processing method. Respondents indicated their level of preference for farmed and wild tilapia and give reasons.	Negative WTP for farmed tilapia. Consumers who indicated not preferred for farmed tilapia justified: “farmed tilapia is not readily available” (83%) and “does not taste good” (11%).
Jonell et al. 2016 [62]	Stockholm, Sweden	Both	General	406.	Self-administered at shopping malls. Survey: frequency of buying eco-labelled seafood, recognition of labels, perceived consumer effectiveness, perceived and objective knowledge of fisheries and fish farming, sources of info, environmental concerns, actors responsible for sustainability, demographics. 5-point Likert scales.	68% sometimes purchase eco-labelled seafood. 78% have a moderate/low perceived knowledge. Maximum correct answer rate of 74% = low actual knowledge. Recognition and perceived understanding of labels was moderate/low, apart from the Swedish KRAV. Main source of info was media (55%). 57% worry about environment impacts of fisheries/fish farms.
Mancuso et al. 2016 [63]	Northern Italy	FF	General	277, representative of Italy population. 127 from local markets (traditional) and 150 in supermarkets (advanced)	Face-to-face interviews (local outdoor markets and supermarkets): fish purchasing habits; consumption drivers; knowledge of overfishing and feed provided; interest in sustainability of fish farming; attitude toward fish produced with insect meals; demographics.	90% are interested in research on more sustainable aquafeeds. 50% are in full agreement, and 40% in partial agreement, with using insect meals. 76% intend to purchase and eat farmed fish even though they are fed on insect meals, so long as the hygiene requirements are met. 7.6% stated they would not buy: 95 % of whom said they feel uncomfortable; 74 % do not trust the production process; 42 % think that product quality could be compromised, 32 % felt it could be somewhat damaged.
Reinders et al. 2016 [64]	France, Germany, Italy, Spain, UK	FF	General	2511 households, (about 500 per country).	Online survey. hypothetical new marine finfish from the European aquaculture, described (e.g. quality, nutrition and sensory, sustainability). Values, costs and risks consumers perceived	3 segments: 1) involved innovators. ≈ subjective knowledge to group 2, but > innovativeness. Older, married, > income, > consumption. Functional, hedonic and ethical values more

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
					about this fish. Consumer involvement, domain-specific innovativeness, subjective knowledge, suspicion of novelties and optimistic bias were measured.	important. 2) involved traditional. Price is important. Does not differ from innovators in intention to buy farmed fish. 3) ambiguous indifferent. did not show any specific interest.
Zhou et al. 2016 [65]	Kentucky, USA	Both	Tuna	421	DCE attributes: production method, form (previously frozen or fresh and never frozen), eco-label (turtle safe, none), price. Survey also questioned purchasing habits, and demographic data.	Participants preferred eco-labelled tuna steak and were likely to pay more for it but were less likely to purchase wild species.
Bronnmann and Asche 2017 [66]	Northern Germany	Both	Salmon	485 retail consumers.	Paper survey, at retail stores. 1) choose preferred salmon product without prior information; 2) Demographics; 3) seafood consumption habits & beliefs; 4) respondents receive information about salmon production, sustainability, and certification (ASC – Aquaculture Stewardship Council & Marine Stewardship Council - MSC), then new set of purchasing choices. DCE attributes: price, production method, certification and processing.	probability of purchase ↓ as the price ↑. Uninformed consumers are less likely to purchase a farmed salmon product. MSC and ASC label ↑ purchase probability, but ASC>MSC.
Froehlich et al. 2017 [67]	Worldwide	FF	General + salmon	n.a.	1596 articles. Headlines pertaining to 'aquaculture', 'marine aquaculture', or 'offshore aquaculture' were extracted using the platform of LexisNexis. Only English headlines were evaluated. Positive, negative or neutral sounding headlines were assigned a 1, -1 or 0, respectively.	Newspaper "aquaculture" headlines have ↑ over time and are overall more positive than negative. Neutral headlines outpaced negative. Sentiment was negative for salmon headlines. "Offshore aquaculture" headlines were more negative. Government collected public comments from the USA and New Zealand mirrored media sentiments; offshore perception being particularly negative in the USA. Public sentiment may be influenced by local environmental disasters not directly related to aquaculture.
Kim et al. 2017 [68]	Florida and South Carolina, USA	FF	General	507, over 18 years of age, seafood eaters, tourists.	Email survey. 1) demographics and trip characteristics; 2) subjective knowledge of aquaculture, attitude toward aquaculture products, intentions of participating in culinary-based aquaculture activities. 5-point Likert scales.	Subjective knowledge and positive attitudes towards aquaculture had a positive relationship with intention to be involved in aquaculture tourism.
Milicic et al. 2017 [69]	Europe	FF	General	635	Online survey with numerical answers, closed questions, semi-closed and open questions and Likert scale from 1 (strongly disagree) to 5 (strongly agree). 1, food buying habits; 2, WTP for certain attributes; 3, knowledge of aquaponics	Attitudes towards aquaponics were positive, with no differences between those who already knew about aquaponics and those who did not. Over 50% had never heard of aquaponics. 17% were

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					and hydroponics (if none, description provided); 3, purchaste intentions of aquaponics products; 4, WTP for aquaponics products; 5, demographics.	WTP up to 40% more for aquaponically produced products.
Murray et al. 2017 [70]	British Columbia, Canada	Both	General	313 grocery shoppers.	Face to face, iPad surveys from grocery stores shoppers. 1) demographics; 2) childhood and adult fish consumption; 3) respondents assessed their agreement with 10 statements on 5-point Likert scale.	52% strongly disagree with "I purchase farmed seafood". 'Sensory qualities' was the most important factor for consumers. Price was in the top 3 most important factors for 47% respondents, and the most important for 12%. The production method was the most important factor for 16% of respondents and was in the top 3 for 35%. Local origin was in the top three for 30% of respondents and most important for 8%.
Pereira et al. 2017 [71]	Algarve, Portugal	Both	Gilthead seabream	105, business consumers of farmed fish	Online survey. 1) fish consumption habits of the organisation; 2) consumption drivers. Importance of 18 attributes were measured on a 5-point Likert scale (1= not at all important and 5 = very important); 3) opinions of a functional seabream; 4) respondents and organisations characteristics.	82% of the total bought fish is wild. 70% claim they do not have enough info at point of sale. Info to be provided in order of importance: production method > capture method > nutrition value > consumer benefits > sustainability > cooking mode. 99% consider external characteristics of fish as important for purchasing; 86% wild fish. Farm-raised fish is not important when it came to buying fresh fish. Value for money is the price item with most importance. Most appreciated factors in functional seabream: "more Omega-3," "more iodine" and "less allergenic". Main reasons for not buying: price, quality, taste, characteristics, bones, and being farm-raised rather than wild.
Popoff et al. 2017 [72]	Edimburgh, Scotland	FF	Atlantic salmon	4 stakeholders + 200 consumers at supermarkets	Semi-structured phone interviews with 4 stakeholders (commercial-scale insect producer; aquafeed producer; Atlantic salmon producer; fish retailer). Face-to-face consumer survey: 1) current fish purchasing decisions, knowledge of farming practices and of fishmeal; 2) attitudes towards insects use in feed and type of waste used for feeding insects; 3) demographics.	Insect producer: growing industry; safety is critical. Aquafeed producer: FM price will ↑; FM can be replaced; raw materials must be safe, approved and price-competitive; insect production limited by small-scale. Fish farmer: room for farming industry to develop; insects would not affect flesh quality; consumers just want food to be healthy, safe and sustainable. Retailer: consumers are interest in food provenance; difficulty in informing through labels; consumer fears novelty; insect/waste type would not be included in label; if research shows positive consumer attitudes, retailers may investigate

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						further. Consumers: limited knowledge of FM composition and production issues. Most were prepared to eat insect-fed fish without concerns. 91% were not aware of this possibility, but no differences regarding awareness and intention to consume were found.
Regnier and Bayramoglu 2017 [73]	France	Both	Seabass and seabream	About 12,000 households. Monthly domestic price series for fish purchased by French households 2007-2012, provided by Kantar WorldPanel	Tested for long-term price parity between farmed and wild products, applying a bivariate cointegration approach to market delineation. Law of One Price test was conducted between price series.	Wild products have higher economic value. Price difference between wild and farmed products is 54% for seabass against 24% for seabream. Fish markets for wild and farmed seabream are partially integrated, yet those for whole wild and farmed seabass are not. The higher price for wild seabass relative to farmed suggests that consumers may be more sensitive to production processes when it comes to higher-value species.
Rickertsen et al. 2017 [74]	France	Both	Salmon, cod, monkfish, pangasius	276, age over 20, representative in average age and income of the general population, main responsible for food purchasing and eat fish over once a month	Financial reward. Sensory evaluation of the species, prepared by a chef. Only cod had wild and farmed alternative. Salmon and pangasius were farmed, monkfish was wild. Participants marked on an unstructured linear scale from 0, "I do not like it at all" to 10, "I like it very much." On the 1st session, consumers only knew the species; 2nd and 3rd, consumers knew origin and production method. After the tasting in 1st session, consumers were asked about their economic valuation of each fish twice, one without knowledge and one after knowing origin and production method. On the other sessions, they were fully aware. Session ended a survey with questions related to the health and safety of the consumption of wild and farmed fish, environmental and welfare aspects, area of origin, attitudes towards the species in the experiment, and socioeconomic status	Consumers perceive wild fish best for safety and health and farmed fish best for environmental sustainability and welfare. They rank salmon the highest on many attributes. Wild fish from the North Atlantic is preferred to farmed fish from France and northern Europe, and farmed fish from developing countries is ranked the lowest. In the sensory trials, salmon received the highest scores, followed by monkfish and cod, while pangasius scored lower.

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Risius et al. 2017 [75]	Germany	Both	Trout (smoked)	Qualitative: 18, fish consumers, not working on fish farming, agriculture, food industry. Quantitative: 447, at least partially responsible for grocery shopping and regular purchasers of fish	Think aloud protocols (qualitative): 6 packages; 3 certified organic labels. Participants chose one while thinking aloud during decision-making. Subsequent in-depth interviews: production method; perceived products' sustainability; evaluation of claims ('environmental friendly produced', 'sustainable aquaculture', 'respecting animal welfare', 'near-natural aquaculture', 'ensuring local employment opportunities', 'saves resources', 'no use of antibiotics') and labels (EU organic, German governmental organic, Naturland, ASC, WWF and a company-owned). DCE: Personal interviews at supermarkets. Attributes: price; origin; sustainability label (ASC, Naturland, fake, no label); claim (from aquaculture, from sustainable production, from natural ponds, no claim). Respondents were told they had to pay for one of the 10 chosen products (at random).	COO most mentioned attribute (preference for Germany). Critics on reduced info on production method in labels. Organic associated with sustainability. Taste was important. Mistrust on labels often mentioned. Sustainable aquaculture associated with 'natural rearing', 'low environmental damage', 'not mass production', 'local origin'. Claims evaluated as imprecise. 'Respecting animal welfare' caused doubts about the feasibility. Fish was seen as being best treated by living in the wild. Some consumers could not imagine an animal-friendly aquaculture production. DCE: high importance of the geographical origin, followed by 'price' and 'claim'. The attribute 'sustainability label' had the lowest impact.
Short et al. 2017 [76]	Minnesota, USA	FF	General	450, age over 18, must visit restaurants and/or grocery stores, must not work at either	Telephone survey. 1) Familiarity with "aquaponics"; 2) Definition provided; 3) perception about aquaponics (agreement with statements, 5-point scale); 4) reasons for not buying aquaponic products; 5) interest in learning more, sources and credibility; 6) demographics.	33% had heard the term aquaponics. Mean answers fall between neutral (3) and agree (4) for all questions. ''Aquaponics operations impact the environment in a positive way'' had the most favourable rating. Main concerns: price and safety.
Tomić et al. 2017 [77]	Croatia	Both	General	1151	Online (90%) and face-to-face (10%) survey. Habits of fresh fish consumption, involvement in cooking (agreement with statements on a 5-point Likert scale), preferences for wild vs farmed fish, physical activity and demographics.	Respondents have neutral (3 out of 5) preference for wild fish vs farmed and taste differences among them. Female, older and > income claim more to detect differences in the taste of wild and farmed. Those from coastal regions and > levels of fish consumption prefer wild fish. > involvement in cooking leads to stronger claims of difference between types of fish and > consumption of wild.
Uchida et al. 2017 [78]	Rhode Island, USA	Both	Salmon and swordfish	340	Seafood auction. Salmon could be wild or farmed. Vouchers were auctioned, with each voucher good for one pound fish from a local supermarket. Images of products were provided. Consumers bid in each round (six rounds) for the three products. After the second round, information was	Without info, wild salmon is valued most, followed by swordfish, and farmed salmon is valued least. Info content had more impact on bids than the source. Info showing that farmed salmon has more omega-3s than wild increases WTP for farmed salmon relative to wild. all five information

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					provided. Treatments: FDA/EPA (environmental protection agency), focusing on risk, no mention of salmon but of swordfish; Industry, focus on benefit, mentions only salmon; University, focus on both but slightly more risks, mentions wild and farmed salmon and swordfish; NAS (National Academy of Science), focus on both but slightly more benefits, mentions wild and farmed salmon and swordfish; Combined FDA/EPA and industry, balanced, mentions salmon and swordfish; no information. Before the auction, each participant was given a $70 allowance. In each round and product, the highest bid wins. Each winner got the product's voucher and leftover cash balance. One round was randomly chosen as the "binding", and the winner won the product.	treatments were equally ineffective in altering consumers' risk perceptions for farmed and wild salmon.
van Osch et al. 2017 [79]	Ireland	FF	Salmon	Age 18-64. 500, representative of Irish population	Online survey. 1) knowledge and attitudes towards aquaculture and IMTA, perception of benefits and threats from aquaculture, questions on marine environmental issues; 2) explanation of IMTA and eco-labels. DCE attributes: production location (Irish waters or outside Ireland), sustainability (increase of 10, 20 or 30% in sustainability due to IMTA, or monoculture production), price; 3) attitudes towards salmon products and purchasing behaviour; 4) demographic info.	9.8% indicated they had heard of IMTA. A follow-up question suggests the true familiarity with IMTA is lower. Aquaculture economic benefits were rated at 4.3 and environmental impact was 3.9 on a Likert-scale of 1–5, (1, "no impact" and 5, "significant impact"). 86% acknowledged economic benefits to IMTA and 80% environmental benefits compared to current production. Most said they used eco-labels as a part of seafood purchasing decisions. Increase of 10% in sustainability has a positive coefficient and the coefficients magnitude ↑ with further increases in sustainability. The Irish values locally produced salmon.
Yip et al. 2017 [80]	Seattle, Portland and San Francisco, USA	Both	Atlantic salmon, king salon, sockeye salmon	1631. Primary or secondary grocery shoppers, consumed salmon more than once over the past year. Controlled for	Online survey. DCE attributes: production method (conventional farming, IMTA, closed containment, wild caught – Atlantic salmon did not have the 'wild' option, Sockeye only had the 'wild' option); eco-certification (generic, none); origin (Canada, USA, Chile, Norway – sockeye and king salmon only had Canada or USA options); price (adapted to species). All wild salmon profiles were	65% preferred wild over farmed salmon, 4% preferred farmed over wild, 29% had no preference, and 2% indicated "don't know". Reasons for preferring wild: more natural, healthier, eco-friendly, better taste. Reasons for preferring farmed: > availability, better taste. 52% not aware of environmental concerns of aquaculture. Low awareness of these farming methods. After description, attitude was more

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				representativeness for each city and for gender proportions.	presented as "previously frozen"; all farmed salmon were presented as "fresh".	positive toward IMTA than closed containment. If salmon produced with them were sold at the same price as conventionally farmed, 38.4% would buy farmed salmon more frequently. Reasons for preferring IMTA: more natural, environmentally friendly and sustainable. WTP a premium of $4.29/lb for IMTA and $1.73/lb for closed containment. 3 classes. 1) wild salmon lovers: ↑ education and income, ↑ consumption. More likely to be concerned about the credence characteristics of salmon and not quality or price. In farming, prefer IMTA. 2) price-sensitive: ↓ income, most likely to concern about price and quality; prefer IMTA. 3) sustainably farmed salmon supporters: ↑ income but ↓ education. More interested in salmon quality. Prefer farmed. Interest in eco-certification.
Ankamah-Yeboah et al. 2018 [81]	Germany: Schleswig-Holstein/Hamburg, Brandenburg/Berlin and Bayer		Trout	610 completed and usable questionnaires. 60% female; mean age 42.	Online survey. DCE attributes: product form (whole fish, filet with skin and bone, filet with skin but no bone, filet without skin and bone), storage form, purchase place (grocery store and specialized fish store), production method (conventional, organic and Aquaculture Stewardship Council - ASC certified), origin, feed (standard feed and insect-based feed) and price. Tasks were introduced with info on the implication of how insects as a fish feed component might reduce pressure on overfishing and competition on protein sources and a description of each attribute.	Highest utility for domestic trout, followed by Denmark and then other EU, relative to Turkey. Use of ecolabels is preferred. 77% are indifferent to the feed.
Antão-Geraldes et al. 2018 [82]	Portugal		brown-trout, rainbow trout	74 students and staff of Polytechnic Institute of Bragança, randomly selected and with no training	Frozen samples were thawed, salted (1.5%), wrapped in foil and baked at 150 °C until internal temperature reached 100 °C. Skin and bones were removed, subsamples of 2x2 cm were presented coded to tasters. Tasters were unaware of the tested species. Overall liking assessed with a 10 cm hedonic scale (completely dislike to completely like).	Sensory quality evaluation revealed all samples were equally well accepted.

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Bronnmann and Hoffmann 2018 [83]	Northern Germany	Both	Turbot	485 retail consumers	Paper survey, at retail stores. DCE: 1) choose preferred turbot product without prior information; 2) demographics; 3) seafood consumption habits and beliefs; 4) respondents receive information about salmon production, sustainability, and certification (ASC & MSC), then new set of purchasing choices. DCE Attributes: price, production process, sustainability certification and processing.	Reasons for not consuming fish: vegetarian, environmental concerns overfishing. Avoided species: tuna, pangasius, eel. Reasons: production method, environmental concerns and taste. Most important attributes: taste, freshness, species. Respondents disagreed that farmed fish has no trace of chemical elements (62%), is healthier (52%), more delicious (48%), and higher quality (51%) than wild. 30/31% are uninformed about fishing methods and endangered fish species. Probability of purchase ↓ as the price ↑. Less likely to buy farmed turbot. MSC label ↑ purchase probability and increased after info. ASC label not significant.
Genschick et al. 2018 [84]	Zambia	Both	General	low-income families. Female respondents only. N=714	To assess whether, and how often, fish was consumed at the household level, including different fish species, small and large fish, and various product types (fresh, dried, smoked, salted).	The poorest households rely on small, dried fish products from capture fisheries while the slightly better off households consume larger, fresh fish, such as tilapia, which are partly supplied by aquaculture.
Hinkes and Schulze-Ehlers 2018 [85]	Germany	Both	Pangasius and tilapia	325, who eat fish and purchase fish products.	Online survey. DCE attributes: species, price, origin, brand, sustainability label (ASC, Naturland, none), fair trade claim (Y/N). Importance of attributes and production process for purchase decision assessed. Knowledge of sustainability standards was self-assessed. Respondents measured concern about a set of sustainability items then selected the biggest concern in aquaculture context. Respondents assessed statements about aquaculture and obstacles for fish consumption.	No clear preference between wild caught fish and farmed fish was indicated. Most were not familiar with standards and their criteria. 10% indicated to deliberately purchase ASC-certified. Respondents are most concerned about food waste, pesticides, animal abuse, and environmental degradation. 10.5% are not concerned about any presented sustainability issues presented, in the aquaculture context. Image of Germany clearly better than Bangladesh and Vietnam in product quality, food safety, social and environmental standard.
Weitzman and Bailey 2018 [86]	Nova Scotia, Canada	FF	General	5 from fish farming, 4 from scientific community, 4 from managing authorities, 4 from NGOs, 4 from wild fishing	Q-methodology. 49 statements grouped into 3 interest themes: potential uptake; benefits and challenges of eco-labels; potential influence of labelling to change aquaculture industry. Semi-structured face-to-face interviews. Participants sorted the 49 statements onto 3 piles based on if they agreed, disagreed, or felt neutral. Participants then sorted cards further onto a 9-point grid chart ranging from − 4 (least agree) to +	4 perspectives derived. 1) optimist: certification benefits many stakeholders; eco-labels improve resource and aquaculture management; they may be a communication tool; many consumers would pay for eco-labelled products; 2) sceptic: not all ecolabels have equal credibility; certification does not guarantee sustainability or necessarily reduce environmental harm of fish farms; standards do not adequately cover specific social concerns

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				industry, 4 from food industry.	4 (most agree), onto a 'forced' quasi-normal distribution (Q-sort). Then, informal interviews to explain choices took place.	such as animal welfare; some consumers would pay for sustainable seafood. 3) pragmatist: overall supportive of ecolabelling; certifications cater to a small demographic of individuals; most consumers will not pay more for labelled products; given the negative public perceptions of fish farming, even with ecolabels, farms would not receive less opposition; confusion as a major challenge. 4) improver: more research is needed to decide if ecolabelling can bring benefits to industry; ecolabelling could benefit several stakeholders but needs improvement; certification may be expensive and not straightforward; consumer confusion by growing number of labels.
Zander and Feucht 2018 [87]	UK, Finland, France, Germany, Ireland, Italy, Poland, Spain	Both	General	4103. Fish consumers, responsible for shopping. Quotas for gender (2/3 female)	Online survey with contingent valuation method. Participants were asked how much they would spend on a fish for an everyday occasion and the number of people they would be preparing it for. They were asked their willingness to pay for fish caught / produced: sustainably, organic, locally, by coastal fisheries, higher animal welfare standards, in Europe or discard free. Questions about perception of European and local production, attitudes toward fish farming, Schwartz values, subjective knowledge, involvement with fish, perceived consumer effectiveness, demographics, were assessed.	Important sustainability aspects in aquaculture for consumers': minimal use of hormones and drugs, protection of endangered species, no environmental pollution. In fisheries: protection of endangered species, no overfishing, recovery of depleted stocks. Domestic production was important to most (particularly in Mediterranean countries), followed by local and European (mainly for Poland and Germany). Greater freshness was the most important reason for geographical preference. Additional WTP: organic (+15%), > sustainably produced (+14%) > higher animal welfare (+14%) >local (+13%) > by coastal fisheries (+12%) > without discards (+10%) >from Europe (+9%). Results vary by country. Overall, 44% are WTP ≈17% more and 9% are WTP ≈43% more.
Zander et al. 2018 [88]	Germany	FF	Trout	Focus groups: 56 fish consumers. Think aloud: 18 consumers (12 female; 50% between 18 and 49 and 50%	Focus groups: What do consumers know about sustainable aquaculture, what do they expect, and what do they know about its labelling? Think aloud protocols: participants were presented 6 packages of smoked trout; 3 organic and 3 from conventional aquaculture. Participants chose one and reported their considerations in the decision-making process by thinking aloud. DCE, in	Consumers' awareness of production methods was low, but they did not demand additional info; they would rather make inferences from their knowledge of agriculture. The attribute "sustainable production" was less relevant for the purchase than freshness, appearance, intended use and price. Most were unfamiliar with RAS, which was less preferred than earth ponds due to

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				older than 50 years). DCE: 459 fish consumers recruited in front of supermarkets.	person, attributes: label (ASC, Naturland/EU organic, fake, none); message (from sustainable production, farmed in natural lakes, from aquaculture); origin; price.	high mechanisation. Surroundings of the farms should be vegetated and nice looking. German origin ↑ probability of choice the most, followed by Danish or Polish origin. Many claims thought to be imprecise. In the DCE, claims “from natural ponds” and “from sustainable production” ↑ probability of choice, but “from aquaculture” ↓. Consumers were unfamiliar with labels; the large number of labels caused confusion and distrust. Some participants want the lowest prices; others associated > quality with > prices.
Ankamah-Yeboah et al. 2019 [89]	Germany (Hamburg/ Schleswig-Holstein; Berlin/ Brandenburg; Bayern)	FF	Trout	1236 respondents.	Online survey. DCE attributes: product form (whole fish, filet with skin and bone, filet with skin but no bone, filet without skin and bone), storage, purchase place, production method (conventional, organic and ASC certified), origin and price. Knowledge of European organic aquaculture production principles was tested (4 multiple-choice questions). After answering, correct answer was provided. Respondents divided in 4 groups: control and 3 info groups. Info was provided about the main reason for the specific production principle. Group 1: “reason for requirements was environmental concerns”; Group 2: “reason for requirements was animal health & welfare”; Group 3: both concerns.	Preference for Germany-produced trout, followed by Denmark, then EU. Most have a preference for organic labelled fish and are willing to pay €1.3/kg more. The organic label has stronger effects than the ASC label. Treatment with info on organic production by focusing on environmental concerns is not significantly different from the control. Providing info on animal health and welfare significantly increases the chance of choosing organic products and increases their marginal WTP. Info focused on both: marginal utility of organic is larger than in the control but smaller than in the animal welfare. It suggests consumers probably know environmental attributes are embedded in the ecolabel and hence feel they are already paying for it once the choice of organic product is made. The info on fish welfare may be new to them, and this is valued.
Banovic et al. 2019 [90]	France, Germany, Italy, Spain and UK	FF	General	1598 (100 participants × 5 European countries x 3 products), fish consumers, responsible for food shopping. Age, gender,	3 product types assessed: fresh, canned, smoked. 3 online surveys, one per product, in each country. DCE attributes: origin; ASC-label (N,Y); nutritional claims (none, rich in omega 3; rich in protein); health claims (none; improves heart function; improves brain function); price. Respondents were asked to imagine standing in the supermarket and selecting which of the	Overall liking did not differ across countries for the three products. Relative attribute importance: COO label > price > ASC eco-label > nutrition > health claims. Claims: nutrition claim “rich in Omega 3” carried the highest utility and was the most attractive across all products and countries.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				income and marital status were balanced across countries and products, considering respective demographic quotas.	products were most/least likely to purchase. Images of products/labels were provided.	
Banovic et al. 2019 [91]	Spain, France, Italy, Germany, UK	FF	Hypothetical new species	Fish consumers (over once a month), main or joint decision makers in grocery shopping; ≈ 500 consumers /country. Quotas on gender and age: 49/51 % of the sample was male/female; age ranged between 18-64 years with mean age 41.2 y.	Online survey. Consumers received a realistic description (and illustration) of a hypothetical new farmed species with improved sensory properties, endorsing all principles of ethical fish production. Subjects indicated if this would be in accordance with their ethical beliefs, what would be their perceptions about quality and value, if they would trust buying this product, and purchase intention. Subjects had to indicate their level of involvement in the study category.	Ethical beliefs affect perceived product quality, perceived customer value and trust in all countries. Perceived product quality affects perceived customer value, which affects trust (more significant in Spain). For French and Italian consumers, the trust construct was mediating most of the effect of ethical beliefs on purchase intention. Consumer involvement strengthens the relationship between ethical beliefs and perceived customer value.
Ferrer Llagostera et al. 2019 [92]	Spain	Both	Gilthead seabream	215 consumers, who had previously consumed gilthead seabream. A quota sampling procedure was used in terms of	Online survey. Subjective (9-point Likert scale) and objective (9 T/F statements) knowledge of aquaculture; perception of the impact on environment (9-point Likert scale). DCE attributes: production method; price; feeding (wild; fishmeal only, fishmeal and vegetable meal, fishmeal and insect meal).	Objective knowledge < subjective (3.8 vs 5.1, out of 9). Consumers perceive a neutral environmental impact of aquaculture (4.8 out of 9). Wild seabream is preferred, and the other 3 regimes have similar preferences among each other. Wild had the ↑ WTP; FM-fed the lowest. Vegetable and insect meal were similar. Wild was perceived as the most environmental friendly, followed by the insect fed. Consumers believe

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				gender and age.		wild to be tastier and insect-fed to be the least tasty.
Flaherty et al. 2019 [93]	Vancouver Island & Maritime Provinces, Canada (West vs East coast)	Both	General	648	Face to face interviews, outside grocery stories, coffee shops and ferry landings. Open-end questions. Assessed: awareness of farmed species, familiarity with aquaculture, impressions of farming systems, information sources, provincial aquaculture and general questions about aquaculture.	Salmon was the most identified farmed species. 84% very or somewhat familiar with aquaculture in the west coast, vs 66% in the east. 51% in the east coast viewed the industry as very or somewhat favourable versus 28% in the west. On the west coast, 74% had very or quite positive impressions of the industry, vs 53% on the east. On the west coast, 56% concerned that salmon farming was harmful/disruptive to wild stocks. Word of mouth was the most common source of info. Overall, 86% felt that aquaculture should be allowed to increase if done in a sustainable way. 55% felt that aquaculture provides high quality seafood that is safe to eat.
Greenfeld et al. 2020 [94]	Australia and Israel	FF	Tilapia (Israel), Trout (Australia)	321 in Australia, 200 in Israel, aware of household shopping habits, representative of the general population in each country.	Israel: respondents were approached in public places and asked to complete a survey. Australia: an online survey distributed by a panel data service. 1) Introduction about aquaponics; 2) Evaluation of willingness to consume lettuce and tilapia or trout, conventional (at standard price) or from aquaponics (at standard price or premium, 3 levels), on a 4-point Likert scale. 3) how much different motivations affect their decisions on food purchase on a 5-point Likert scale; 4) demographics.	Most in both countries said they would not increase consumption of lettuce/fish if aquaponic produce were available even at a similar price as a non-aquaponic. Consumption was negatively correlated with prices for lettuce and fish in both Israel and Australia. A segment of consumers in both countries are willing to consume aquaponic products at a premium.
Guney 2019 [95]	Turkey (İstanbul, Ankara, İzmir, Trabzon, Adana, Van, and Gaziantep)	Both	General	526 consumers, responsible for their household or family purchases	Face-to-face interviews. 1: consumers evaluated statements of fish in general and farmed/wild fish in general, with a 5-point Likert scale. 2, demographics.	72.9% prefer farmed fish. Most concern about stocks depletion and agree that wild are fresher and more expensive. Positve correlation between finding fish preparation easy and consuming farmed fish. Those who value fish variety > likely to buy farmed fish. Those who value fish origin and who believe wild fish is fresher < likely to buy farmed fish. > age, < likely to buy farmed. < education, > likely to buy farmed fish.

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Hilger et al. 2019 [96]	San Francisco, USA	Both	General	10 retailer stores	Scanner data from a regional upscale supermarket retailer. Seafood products included in their label a "traffic light" sustainability level: red, yellow or green depending or their environmental impact. 8 stores of the retail did not implement the system (controls), 2 did (treatment). Weekly price adjustments were not connected to the label. Store traffic was also measured.	Consumers prefer green products relative to yellow products and they dislike red products relative to yellow products. Consumers react more to yellow products. Preference for wild and USA seafood.
Hynes et al. 2019 [97]	Ireland and Norway	FF	Salmon	959 Irish, 1001 Norwegian. >18 of age. Quotas based on population census.	Ireland: face-to-face interviews; Norway: phone interviews. 1) attitudes towards marine environment and aquaculture; 2) fish eating habits; 3) Which salmon they would be more likely to buy: conventional for €15.50 /kg (~€2.75/pax) or an eco-labelled salmon for€X_1 /kg (~€X_2/pax). 6 paired bid values for X_1 and X_2 were used in surveys (1 option per suvey). 4) demographics.	Environmental credentials are an important driver of purchase decision. However, the high joint probability in Ireland for considering environmentally friendly practices important but not being WTP a premium and in Norway for not considering environmentally friendly practices important but being WTP a price premium suggest that there are other incentives.
Kulesz et al. 2019 [98]	Sweden	Both	Salmon	A representative sample of 1005 Swedish consumers of fish (over twice a month), selected from a Swedish consumer panel provided by the marketing company GfK NORM. Excluded those working in fish-related industries.	Online survey. 1) demographics; 2) general attitudes to the impact of science on food quality and the environment; interest and perceptions related to fish consumption. 3) DCE attributes: production method; sterilization technique (none vs hormones vs triploidization); price. The DCE was preceded by a background text. It either simply described triploidization or further characterized it as being a biotechnology technique.	Consumers who were not informed about the use of biotechnology evaluated farmed fish positively and were not WTP over 18 SEK/kg for wild. Coefficients for sterilization were negative, meaning consumers prefer untreated fish. Female consumers > WTP for wild. People living in larger cities > likely to buy farmed. When "biotechnology" was included, variation in WTP ↑.
Lee and Nam 2019 [99]	South Korea	Both	Black rockfish; red seabream	766 Households heads or home-keepers; age 20-69	Phone interviews. Survey: 1) questions about the preference for live fish; 2) questions on consumption behaviours; 3) socio-economic characteristics.	Respondents exhibited little difference in preference between aquaculture and wild products, because of the diffusion of aquaculture products.

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Quagrainie 2019 [100]	USA	Both	Striped bass	581; over 18 years; familiar with the seafood consumption of household	Online survey; interest and willingness to pay for midwestern saltwater seafood and striped bass (respondents chose from defined price categories), seafood preferences and purchasing attitudes; demographics.	31% of respondents would be WTP up to \$3.99/lb; 23% would pay \$4-\$4.99/lb; 18% would pay \$5-\$5.99/lb; 28% would be WTP ≥\$6/lb, for midwestern striped bass. 34% prefer wild fish; 16% prefer farmed fish; 42% were indifferent.
Ramalho Ribeiro et al. 2019 [101]	Portugal	FF	Gilthead seabream	778	Online survey. 1) fish consumption and preferences. 2) acceptance of farmed fish and fish fortification: consumption of farmed fish; importance of knowing fish origin (5-point importance scale); choosing 2 out of 7 attributes that could be improved in farmed fish, with a 5-point scale; agreement on fish fortification through feeding, with beneficial nutrients for the human health, (5-point scale); identifying 2 nutrients (out of 8) suitable for fortification. Brief description of the beneficial effects was provided. 3) willingness to consume fortified fish; buying preference at no price change; willingness to pay a higher price for fortified fish (Y/N). 4) demographics.	Freshness, flavour and quality were the most important attributes, ahead of price. Men were more favourable than women to consume farmed fish. The older groups were more receptive to farmed fish. Most selected flavour, more diversity, nutritional value, fat content and texture as the main desired improvements in farmed fish. 52% agreed with fish fortification. 49% showed willingness to consume fortified fish, mainly among the oldest (90%) and youngest (68%). omega-3 fatty acids and antioxidants were the most popular nutrients for fish fortification. 55% are WTP the same price as conventional farmed; 35% at a higher price.
Reig et al. 2019 [102]	Barcelona, Spain	Both	General	30, for the nominal group. Consumers: fish consumption at least once a month; involved in food buying. Fishmongers: independent shop, must sell wild and farmed. 14 experts from the consumer, fishmonger and wholesale sectors.	1) nominal group technique. Groups: regular consumers, occasional consumers, fishmongers, wholesalers. Two rounds, one for positive aspects of aquaculture, one for negative aspects. Participants wrote their ideas, presented them, ideas were ranked, debate, new ranking. 2) Delphi survey. Statements from the previous phase were used to make the statements for the Delphi phase. Two-round questionnaire. Experts provided their agreement with each statement, in a percentage rating scale. Then, all info collected with the mean and the standard deviation for each statement and the individual answers to each statement, were presented and a second round took place.	Occasional consumers: positive aspects of aquaculture compared to fisheries was the low price. Negative aspects, farmed fish undergo non-natural growth and worse taste. Regular consumers: positive aspects, low price, acceptable taste and higher health control. Negative aspects, uncertainty and lack of information, and the use of chemicals for promoting growth. Fishmongers: positive aspects all-year round availability and high diversity. Negative, lack of trust in farmed fish, lack of info and knowledge from consumers. Wholesalers: Positive aspects, buying convenience and price stability. Negative, lack of info on feeding, treatments and genetic modification. Experts: statement with the highest consensus related to price stability and affordability of aquaculture products. Statement with the maximum dissensus

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						was that aquaculture has more sanitary control than fisheries.
Richard and Pivarnik 2019 [103]	Rhode Island, USA	Both	General	968, age above 18	Mail survey. 1) demographics; 2) behaviour and purchasing habits; 3) interpretation of local seafood: which species are considered to be local, recognition of the local logo, impact of logo and other topics on purchasing habits (5-point scale), self-knowledge (4-point Likert scale) about seafood quality and safety; 4) attitudes (5-point Likert scale) and sources of info (check all) about local seafood.	Most would prefer local seafood if they could easily find it and trust the brand. Quality, taste preference, safety at purchase and absence of contaminants were important or very important factors for purchasing. Respondents felt only somewhat knowledgeable about key attributes. 53% would be more willing to try seafood if it were labelled local. 68% interpret "local" seafood as being caught within the state waters, while 55% as farm-raised in state waters.
Risius et al. 2019 [104]	Germany	FF	Trout (smoked)	447. Fish consumers, responsible for food purchasing	Personal interviews at supermarkets. DCE attributes: price; origin; sustainability label (ASC, Naturland, hypothetical, no label); claim (from aquaculture, from sustainable production, from natural ponds, no claim). Realistic market conditions applied (prices for Turkish always lower than Danish or German). To reduce hypothetical bias, respondents were instructed that they would have to pay for one of the 10 chosen products (at random).	5 consumer classes: 1) average. Mostly influenced by COO (Germany and Denmark preferred) and price. Prefer the 'sustainably produced' and 'from natural ponds' claims and the hypothetical label. 2) COO oriented (prefer Germany). Price, label and claim not significant. 3) Sustainability oriented. Label and claim most important. Prefer Naturland label, and 'from natural ponds' / 'sustainably produced' claims. Rejected 'from aquaculture' claim. Price not significant. 4) premium-price oriented. Focus on medium -high prices (quality cue). Weak importance of COO (Germany preferred), label and claim. 5) Low-price oriented. Claims important (prefer 'from natural ponds'). Label not significant. Preferred COO was Denmark (Germany negative preference).
van Osch et al. 2019 [105]	Ireland, Israel, Italy, Norway, UK.	FF	Salmon or seabream	2520 (~500 per country). Stratified according to age, sex and region proportions in each country, to ensure a	Online. Background on what IMTA was and how it operated. 1) perception of the aquaculture industry and marine environmental problems; 2) seafood consuming behaviour, focusing on use of ecolabels to make informed purchasing decisions; 3) DCE attributes: production location (national waters or outside), sustainability (increase of 10, 20 or 30% in sustainability due to IMTA, or monoculture production), price. Farmed salmon	14% indicated they had heard of IMTA. A follow-up question asking for a description of IMTA suggests the true familiarity with IMTA is lower. In a scale 1-4 (always-never), use of ecolabels was 1.9. WTP is positive for all attributes and countries, indicating that the public is WTP a premium for products produced in a more sustainable method such as IMTA. WTP a premium for seafood produced in national waters.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				representative sample	for northern Atlantic countries and farmed seabream for Mediterranean; 4) demographic indicators.	
Yeşilsu et al. 2019 [106]	Turkey	Both	General	472	Survey in restaurants. Preferring to consume farmed or wild fish; age; gender; region (coastal vs interior); income; fish consumption frequency; asking the origin of fish at a restaurant; paying attention to origin in processed fish; willingness to pay and to consume farmed fish labelled without drugs (antibiotics) residues.	56% claim to always ask about the origin of fish eaten at restaurants. 51% do not care about the origin of processed fish. 36% are not WTP more for antibiotic-free farmed fish; 25% are WTP 10% more and 22% are WTP 20% more. > age, less likely to buy farmed fish. Those who consume fish more frequently tend to prefer wild fish.
Yi 2019 [107]	South Korea	Both	Loach	525; online market survey company was used to collect a demographically balanced sample	Online survey. 1) attitudes and subjective norms; 2) production description, followed by "are you willing to pay *random value* for rice/fish?" If yes, consumers were asked if they were willing to pay twice the random value. If no, consumers were asked if they were willing to pay half the random value. 3) demographic info.	The WTP for 1 kg of live IAA fish (i.e., loach) is \$26, around \$6 > than for the conventional aquaculture product. Only social norms are statistically significant in affecting consumers' WTP
Yi 2019 [108]	South Korea	FF	General	960, balanced sample in terms of gender, region, and age.	Online survey. At the beginning, description and image of the ASC were given to. 1) demographic info. 2) attitude, subjective norm, perceived behavioural control, intention to purchase ASC-certified products. 3) environmental awareness.	All latent variables (attitude, social norm, and perceived behavioural control) positively affect consumers' behavioural intention. Social norm has more influence on behavioural intention than other constructs. High environmental awareness group: attitude influences more than social norm. Low environmental awareness: social norm influences more than attitude.
Abdikoglu et al. 2020 [109]	Tekirdag, Turkey	Both	General	270 households.	Face to face surveys. Preferences about fish consumption of the consumers in Tekirdag were grouped by factor analysis and the effects of the factor groups and socioeconomic characteristics of households were determined with the Tobit model.	Most effective factor in purchase decision was attitude towards processed products. Other factors: conscious purchasing, opinion about aquaculture, health benefits. > education level and knowledge about fish → > fish consumption.
Alam and Alfnes 2020 [110]	Dhaka, Gazipur, and Mymensingh, Bangladesh	Both	Rui, catla, pangasius, singi/magur	410; responsible for over half of food purchasing. Mean age, household size and income are representative	Personal interviews. 1st part: socioeconomic characteristics, household consumption patterns, attitudes and preferences regarding fish; 2nd: DCE. Attributes: price, production method, origin, freshness, species. Consumers rated the importance of 14 attributes of farm-raised and wild fish on a 7-point Likert scale.	Freshness and taste were the most important attributes for farmed and wild fish. Fat, bone content, and origin were the least important. Price was the 8th most important. Availability was the 4th most important attribute for farmed fish and the least important for wild. 61% were concerned about the negative environmental impact of fish farming and 56% about the overfishing of wild

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				of Bangladesh households.		fish. 63% were concerned about farmed fish welfare and 52% about wild fish welfare. No demographic variables were significant. Consumers are not willing to pay (WTP) > for wild-caught. Perception of farmed Pangasius was negative (49%).
Antão-Geraldes et al. 2020 [111]	Portugal	Both	brown-trout, rainbow trout	74 students and staff of Polytechnic Institute of Bragança, randomly selected and with no training	Frozen samples were thawed, salted (1.5%), wrapped in foil and baked at 150 °C until internal temperature reached 100 °C. Skin and bones were removed, subsamples of 2x2 cm were presented coded to tasters. Tasters were unaware of the tested species. Overall liking assessed with a 10 cm hedonic scale (completely dislike to completely like).	The panel of consumers equally well accepted all samples.
Chikudza et al. 2020 [112]	Denmark, Italy, Ireland, Germany, Greece, Norway, Poland, Spain and the United Kingdom	FF	General	26 participants, producers and participants in the chain of custody, involved with ASC label	Q-methodology. 30 statements regarding incentives and challenges of ecolabels were provided through email and\or a paper survey and ranked by participants, from mostly disagree (−4) to mostly agree (+4), and five statements were placed on neutral. This was followed by a phone, personal or online interview to further understand perceptions.	Producers claim that adopting ecolabelling has benefits such as enhanced market access, product acceptance, price premium, long-term supply contracts, increased investment attractiveness, positive consumer perception of aquaculture products and increased reputation. Identified challenges: as compliance costs, costly annual audits and lack of long-term benefits.
Garlock et al. 2020 [113]	USA	Both	red drum, longfin yellowtail, tilapia, red snapper	Wholesale buyers (unknown n)	DCE attributes: species; origin; production method; product form; price. Respondents were asked about their attribute's preferences in finfish in: high-end retail and white table cloth restaurants and mid-range retail, chain and family restaurants. Perceived preferences for production method and origin in high-end and mid-range retail were measured using a Likert-type scale.	Buyers prefer fish produced in the U.S. over imported fish. Wholesale buyers do not strongly prefer wild over farmed. It is more probable that Gulf farmed fish will substitute for imports than for domestic wild-caught fish given wholesale buyers have a preference for domestic fish to imported fish, but no significant preference for wild-caught fish to farmed fish.
Hartmann and Siegrist 2020 [114]	Germany	Both	General	973, with a 50% male/female quota and equal number of respondents in each age group	Online survey with financial reward. Participants read short descriptions of the meat/fish production methods including their downsides relative to animal welfare. Participants rated each from 'morally justifiable' (1) to 'morally not justifiable' (8). Only the extremes were verbally labelled. No middle option was provided. Then, justifications	No differences were observed in how morally justifiable respondents perceived farmed fish and wild fish production.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				from 20-80 years	for eating meat and fish were assessed on a 1-9 scale of agreement. Meat and fish consumption were questioned. Participants indicated the % of their usual meat consumption that they would be willing to substitute with alternative protein sources	
Hoque and Alam 2020 [115]	Bangladesh	FF	General	498, responsible for fish purchasing at home.	Personal survey: 1, subjective and objective knowledge (T/F statements); 2, attitudes and purchase intentions; 3, sociodemographics.	Consumers' subjective knowledge is significant for purchase intention while objective knowledge is not. Consumers' subjective and objective knowledge, knowledge discrepancy and confusion have no influence in forming consumers' attitude towards farmed fish. Consumers who overestimate their actual level of knowledge hold negative attitude towards farmed fish and vice versa.
Kitano and Yamamoto 2020 [116]	Japan	Both		532 adults responsible for purchasing over 50% of the fresh food for home consumption.	Online survey. 1, relative evaluations of various protein sources; 2, annual purchase counts of the species; 3, sociodemographics; 4, eating habits; 5, psychological questions related to fish consumption; 6, knowledge related to fish and the fishing industry; 7, fish and fishing-industry-related experience.	Wild fish are rated higher than farmed fish in terms of “quality” and health properties. Japanese consumers place particular importance on “availability”
Menozzi et al. 2020 [117]	France, Germany, Italy, Spain, UK	Both	Salmon, trout, seabass, seabream, pangasius, herring and cod	2433 fish consumers (≈500 per country, representative of the national populations in at least three of the following: age, gender, educational level, and geographical macro-areas).	Online DCE. Attributes: price, production method, presentation (whole, fillet, ready to cook), eco-label, nutritional claim. 9 blocks of 8 choice sets with 7 product profiles plus the “no choice” option.	Higher premiums for wild than farmed fish. Positive premiums for a sustainability label and nutrition and health claims, with high heterogeneity across countries and species.
Pulcini et al. 2020 [118]	Italy	FF	General	7795, aquatic food consumers.	Online survey. 1) demographics; 2) habits of aquatic food consumption with focus on farmed fish; 3) knowledge and awareness about organic	75% of family units buy < 2 kg of farmed aquatic food per month, spending 20–50€. > age consumes < farmed fish. 40% never consume

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
					aquatic food. Brief explanation of terms used provided.	organic aquaculture products because of lack of knowledge, unavailability, and high price. 55%, regardless of age, sex, and area, were WTP a premium for organic fish, seen as antibiotic free, traceable, and sustainable.
Ruiz-Chico et al. 2020 [119]	Cadiz, Spain	Both	General	558, adults	Face to face surveys in shopping centres. 1) fish consumption habits; 2) knowledge; 3) views of aquaculture vs wild; 4) demographics.	39% consumed farmed fish at home; 29% doesn't know the origin. 52% does not know the origin of fish eaten away from home. 43% were very or fairly satisfied with farmed fish; 20% were not very satisfied or not satisfied at all. Support in men > women. Perception improved with income. Main advantages of aquaculture: economic improvement, more affordable prices, and a greater variety and quantity of fish throughout the year. Disadvantages: 56% said 'none'. 20% said 'misuse of feed and chemicals'. 52% believe aquaculture in their area should increase. High uncertainty about social aspects of aquaculture. Most believe farmed fish is cheaper, more common in supermarkets, with lower variety, less environmentally friendly, unhealthier, lower quality and worse taste.
Ruiz-Chico et al. 2020 [120]	Spain (western Andalusia and Mediterranean area)	Both	General	803, adults	Face to face surveys in shopping centres. 1) Buying behaviours; 2) knowledge; 3) views of aquaculture vs wild; 4) impact on the environment.	37% consumed farmed fish at home; 31% does not know the origin. 52% does not know the origin of fish eaten away from home. 43% were very or fairly satisfied with farmed fish; 19% were not very satisfied or not satisfied at all. Perception improved with income. Main advantages of aquaculture: economic improvement, cheaper prices, and a greater variety and quantity of fish. Disadvantages: 50% said 'none'. 17% said 'abuse of feed and chemicals'. 45% believe aquaculture should increase. Men were more positive. Aquaculture seen as having cheaper prices and greater fish quantity to markets. Wild fishing had more variety and a better quality, being healthier and more environmentally friendly.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
Ruiz-Chico et al. 2020 [121]	Andalusia, Spain	Both	General	579, adults	Face to face surveys. 1) Buying behaviours; 2) knowledge; 3) views of aquaculture vs wild; 4) impact on the environment.	45% were very or fairly satisfied with farmed fish; 19% were not very satisfied or not satisfied at all. Perception improved with income. 51% believe aquaculture should increase. Men were more positive. Main advantages of aquaculture: economic improvement, cheaper prices, and a greater variety and quantity of fish. Disadvantages: 55% said 'none'. 20% said 'abuse of chemicals'. Aquaculture seen as having cheaper prices and more availability. Wild fishing had more variety and a better quality, being healthier, tastier and more environmentally friendly.
Yang et al. 2020 [122]	Canada	Both	Arctic char	1342, fish eaters, representative of Canada in age and gender.	Online DCE. Attributes: origin (Canadian arctic, B.C., Alaska or unstated), production method, producer (indigenous or non-indigenous fishers or non-stated), certification (geographic origin, sustainability, authenticity, none), price.	On average, consumers may not consider the presence of certification for origin or authenticity to be important when purchasing Arctic char. Consumers have a strong preference for Arctic char produced Canada (vs Alaska). consumers strongly prefer wild char to farmed. Consumers prefer to be assured about the sustainability of Arctic char.
Allegro et al. 2021 [123]	Sicily, Italy	Both	General	1,698 respondents.	1, consumption of fish/seafood at home; 2, perception of fish/seafood; 3, social-demographics. Interviews were carried out by a private company via Computer Assisted Personal Interview.	Respondents that ate fresh fish less often justified it with "costs too much" (28%), "lack of habit" (22%) and "too much time and work to prepare it" (22%). Price was an important factor for purchasing for 79%; the most important for 34%. Species was the most important factor for 24% and production method for 21%. 86% consumed aquaculture products ≥1x/month. For those with low aquaculture consumption, 30% said it was due to "lack of habit", 29% said they prefer wild fish. Most were unaware of which fish products were farmed. <30% disagreed farmed fish was at the same levels as wild on nutrition, taste, naturalness, quality, safety, delicacy. 49% partially agreed that aquaculture has a negative environmental impact. 56% partially agree that fish farming guarantees animal welfare.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
Asche et al. 2021 [124]	Germany		Pangasius, tilapia, trout fillets	Market data from Homescan panel on food purchases collected by GFK. 10,538 different households.	Households scanned their daily food purchases using 'European Article Number' codes, identifying each purchased product. Info about the point and date of purchase, product characteristics, and if there was a promotional sale. Development of a hedonic price model.	Pangasius and tilapia products sold with the ASC label fetch a price premium of 6% compared to unlabelled, while trout fetches a premium of 9%. The premium tends to decrease as the price level of the retailer or brand increases.
Bazoche and Poret 2021 [125]	France		Rainbow trout	301 adults, involved in food purchasing, without food allergies, with internet access at home and having made online purchases.	Online survey. 1, sociodemographics and fish-eating habits; 2, perceptions of fish food in the wild and in farming; 3, perceptions of eating insect-fed trout; 4, intention to consume this product. 50% of respondents were provided information regarding the environmental benefits of insect based aquafeeds.	76% of informed participants would be ready to eat insect-fed fish against only 64% of uninformed participants. Being informed, gender and food neophobia affect acceptance of fish fed insects.
Budhathoki et al. 2021 [126]	Denmark	Both	Salmon (smoked)	92 untrained fish consumers.	Consumer survey with taste testing of smoked salmon under blind and informed condition, followed by focus group discussions. Survey: 1) demographics, fish consumption habits, attitude towards fish production and labels, 2) overall liking and liking of sensory attributes (colour, smell, taste, firmness, juiciness) in a 7-point Likert scale. Samples: one wild caught, one conventional farm-raised; one organic farm-raised. Focus group: 2 sessions, 5 participants each. One session with light consumers, another with heavy consumers.	34% did not have previous preference for either wild or farmed, while 30% preferred wild. Consumers strongly agreed about labels usefulness to determine product quality. Liking of sensory attributes and overall liking of organic salmon ↑ after info, but not significantly. Liking of 'taste', and 'firmness' of conventional salmon significantly ↓ and the liking of sensory attributes 'colour', 'taste', 'juiciness', and overall liking of wild significantly ↑ after info. Taste is the main contributor to liking. Blind condition: overall liking of both farmed samples > than wild and similar among them. Informed: overall liking of organic > conventional farmed; wild was similar to either. Focus group: consumer beliefs related to food safety, animal welfare and sustainability & purchasing habits influence smoked salmon preferences.
Cantillo et al. 2021 [127]	Gran Canaria, Spain	FF	Seabream and seabass	351. Adults; responsible for buying food;	Online survey. 1) consumption habits; 2) importance (9-point scale) of 16 attributes regarding last purchase of seabass/seabream	Most important: hygiene and food safety; health; and freshness. Less important: availability, bones, consumption by friends/family. Highest

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				consumers of these species; associated with the University of La Palma	(related to health and nutrition, safety, sustainability, sensorial characteristics, convenience, social behaviour and price). 3) 10 best-worst case tasks: respondents selected the most and less important and "satisfied with" attribute out of 4. 4) demographics.	satisfaction: same as most important. Lowest satisfaction: bones, consumption by friends/family, habit since childhood. Best-worst scale: bones, size of product and habit since childhood are less important/satisfactory. Health, nutrition, flavour and freshness are more important /satisfactory. Hygiene and safety are important, but less satisfactory (100% vs 82%).
Carrassón et al. 2021 [128]	Spain	FF	General	300 consumers that purchased and consumed seafood more than once in the past 2 months	Online survey: 1) demographics; 2) attitudes and opinions about aquaculture. Then, information was provided in either text format or docuweb format. Section 2) of survey repeated. Assessed: weekly consumption/expenditure; species; production; knowledge of aquaculture definition (agreement with sentences, Likert scale 0-10).	Before info: overall perception of aquaculture was 5.6/10. Most positive considerations: providing essential nutrients, healthy, affordable. Lowest scores: trusting aquaculture processes, being informed about them, tending to accept. > age, > acceptance of aquaculture. Agreement scores ↑ after info. Type of communication did not affect overall score. Consumers with less previous knowledge change more their opinion.
Hoque 2021 [129]	Bangladesh	Both	Tilapia	490, older than 21 and responsible for fish purchasing at home.	1) DCE attributes: water efficiency; feeding; label; price. Attributes were explained. 2) attitudes towards sustainability and farmed fish (7-point Likert scale). 3) demographics. 4) importance of environmental, biological, safety and economic indicators on choices (ranking).	Perceived knowledge of sustainability is average. Attitude towards farmed fish is positive. 41% ranked environmental indicators as most important for decision. For low water consumption, consumers are WTP the highest premium among the sustainability indicators. Low water consumption and appropriate feed increases their utility. Eco-label decreases utility. 4 classes of buyers: 1, opted out/ wild fish buyer; 2, averagely-sustainable fish buyers; 3, intermediately-sustainable fish buyers; 4, fairly-sustainable fish buyers.
Loncaric et al. 2021 [130]	Croatia	FF	General	123, responsible for food purchasing	Online survey. 1) attitudes towards farmed fish; 2) subjective norms; 3) perceived behavioural control; 4) intention to consume farmed fish. All items measured in a 5-point Likert scale.	Main reasons for not eating fish: do not have the habit; do not like smell/taste. Moderately positive attitudes towards farmed fish (mean 3.2-3.6) and intention to consume farmed fish (3.2-3.5). Slightly negative for satisfaction when farmed fish is on the menu. Most disagreed that their family/friends think they should consume farmed fish, but they believe that they consume it. Most disagree that the purchase of farmed fish is a good choice.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
López-Mas et al. 2021 [131]	France, Germany, Italy, Spain and UK	Both	General	2511 (~500 /country). including quotas for gender (50% women) and age (18-64 years). Responsible for food purchases and preparation and frequent consumers of both or at least one type of fish (wild or farmed).	Online survey: 1, beliefs: 19 items, in the format 'wild/farmed fish _____ than farmed /wild fish', 7-point Likert scale; 2, objective (T/F/don't know) and subjective (7-point Likert scale) knowledge. 3, wild/farmed fish consumption; 4, sociodemographics.	Most had average subjective and objective knowledge. Consumers believed wild fish > quality than farmed, but that farmed was > in control, price, and availability. > consumption of farmed fish reported, positive perceptions of products do not necessarily drive consumption. Consumers believed that farmed fish were < fresh and contained > antibiotics than wild. Five clusters: pro-wild fish, slightly pro-wild fish, balanced view, open to aquaculture, and pro-aquaculture. Males had > consumption of wild, and the > education, the > the farmed fish consumption.
Malcorps et al. 2021 [132]	Worldwide	FF	General	Seafood exhibitor booths at business-to-business seafood shows. 229 in Boston, 227 in Brussels, 117 in Guangzhou, 261 in Qingdao and 150 in Shanghai.	Investigation of which logos, certifications, and claims were presented at the exhibitor booths within seafood trade shows. Passive survey: data collected from text and logos observed at exhibitor booths. The following data were collected: country of origin, companies' activities, followed by observations of booth contents aimed at identifying messaging in the form of logos and/or words displayed at the exhibitor booths. Focus was on messages related to sustainability (socioeconomic and environmental spectrum), quality, health characteristics, food safety, and provenance of the product.	The Sustainability/Environmental messages were more present at the Boston and Brussels shows than at the Chinese shows. Asian and Chinese exhibitor booths across Boston and Brussels shows had a relatively high presence of the ''quality" logo category, whereas for Asian exhibitor booths in Guangzhou and Qingdao, and European and NA exhibitor booths at Qingdao, the use of "quality" words compared to logos increased. Overall, the category "safety" was most commonly shown at the Chinese seafood shows and Chinese and Asian exhibitor booths across all shows
Meng et al. 2021 [133]	Shanghai, China	Both	General	394, responsible for aquatic product purchasing at home.	In-person survey at supermarkets: 1) monthly expenditures on aquatic products; 2) experience with safety and quality of aquatic products, preferences for product certification and inclinations to pay for quality and safety assurance; 3) demographics.	86% would pay more for quality. 70% would pay more for an organic product. 64% would pay for certified aquaculture fish products. 41% would pay premiums for wild fish.
Mitra et al. 2021 [134]	Bangladesh	Both	General	138, who buy capture and culture fishes frequently	Interviews at fish market. Consumer perceptions for capture and culture fish were measured with agreement with 22 statements (5-point Likert scale).	Wild fish was preferred because it was "free from antibiotics, hormones and chemicals", better taste, safer and more nutritious than culture fish. Wild fish seen as more expensive. Those purchasing exotic fish are more likely to prefer

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
						culture fish. Probability of preferring wild fish ↑ with its average price. Probability of preferring wild fish ↑ with the number of family members.
Runge et al. 2021 [135]	Wisconsin, USA	FF	General	548, responsible for food purchasing	Mail survey. 1) general food consumption; fish consumption; places for purchasing; 2) attitudes towards fish and food preparation; 3) food consciousness and trust in system actors to keep fish safe; 4) attention to different news topics about food production; 5) demographics.	Perceptions of fish farming as environmentally friendly were significant. Respondents had higher trust in fish farmers to keep fish safe if they trusted government agencies to do the same. Attention to environmental news and conservative ideology were positively related to trust in fish farmers, and a preference for GMO-free/organic foods was negatively related to the same.
Sagun and Sayğı 2021 [136]	Turkey (coastal regions)	Both	General	650 households	1) demographics; 2) consumption of fish products and consumption priorities; 3) reasons for not consuming fish products; 4) consumption frequency; 5) aquaculture; 6) why consume fish; 7) where they buy fish; 8) what they pay attention to when buying fish; 9) where fish is consumed; 10) preferred cooking method.	62% have bought aquaculture products. Main farmed species: seabream, seabass, trout. 84% prefer wild to farmed. 86% believe there is a difference between farmed and wild fish. Aquaculture perceptions: emphasis on its preferences due to being healthy, practical, cheap, quality and environmentally friendly. Factors determining purchase: product freshness (39%), visual appeal (34%), price (18%) and others (9%; trust, easy cooking, promotion and taste).
Sajiki and Lu 2021 [137]	Japan	Both	General	1000, 20 years old or above, responsible for seafood purchasing	Online survey. Best-worst scale analysis. 11 items (origin, price, appearance, ease of cooking, freshness, seasonality, nutrition, taste, wild origin, environmental attributes, safety) that are considered important when purchasing raw fish were considered for a choice set. In each set, 5 attributes were presented, and respondents chose the most and least important ones.	Consumers prioritize freshness, price, taste, appearance, safety, seasonality, ease of cooking and eating, nutrition, origin, wild fish (vs farmed), and effect on natural environment and ecosystem, in that order.
Suarez-Caceres et al. 2021 [138]	Spanish speaking countries	FF	General	636	Online survey. 1) demographics; 2) previous knowledge on aquaponics. If they did not know the concept, brief introduction presented; 3) product preferences, willingness to be an aquaponic producer, main diet and purchase habits, if they produced food for self-consumption, reasons for buying aquaponic products; 4) preference for purchasing at the same price conventional, organic or aquaponic products; 5)	58% knew what aquaponics was. 90% reported aquaponics to be a quite or very interesting type of production. 89% were willing to consume aquaponic products. Motivations: quality, taste, concern for chemicals. 48% would prefer aquaponic or organic products over conventional. 30% are indifferent. 32% would pay the same for aquaponic and conventional products. 11% would pay up to 2% more, 20% up to 5% more and 18%

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					willingness to pay for aquaponic products (9 options, from 'never buying an aquaponic product' to 'willing to pay a price 30% higher than a conventional product').	up to 10% more. 12% would buy aquaponic products with a price more than 10% higher.
Tezzo et al. 2021 [139]	Yangon, Myanmar	Both	General	13 households. total of 46 people. Urban migrants.	Personal interviews. Semi-structured questionnaire: 1) migration and life histories; 2) general everyday food practices; 3) fish eating practices; 4) fish cooking practices; 5) fish sourcing practices. When possible, participating households were observed during their shopping excursions and/or while preparing and eating their meals.	Freshness is the main value indicator Farmed fish are often purchased by those who value year-round availability. Wild fish preferred; farmed fish is only purchased because of high price of wild. Farmed fish quality is more difficult to discern than wild. Younger migrants tolerate some farmed species into curries (traditional dish), mrigal or rohu. Inappropriateness of other farmed species such as tilapia. Farmed fish more suitable for frying. This increases consumption of farmed fish, due to practicability and acceptability by children. Cheaper fish paste processed from farmed fish, more available than made from wild, is not of an 'acceptable' quality. Farmed fish also used for steaming during celebrations with many people (due to the need for high quantities).
Witter et al. 2021 [140]	Canada	Both	General	2006, geographically diverse sample representing multiple consumer markets across Canada	Online survey: 1) seafood purchasing behaviours; 2) attitudes and opinions about seafood features; 3) WTP for these seafood features; 4) demographics.	Reasons for not consuming any seafood: disliking the taste (57%), expensive (23%), health reasons (22%), environmental impacts (19%), inconvenient preparation (11%), lack of availability (5%). 32% say that taste, appearance, and/or freshness was the most important; affordability (20%); accurate product info (17%). Less selected as most important: sustainability (5%), production method (wild/farmed) (5%), local origin (4%), convenience of preparation (3%). Very few disagreed with preferring wild over farmed seafood, but several neither agreed nor disagreed. Product quality (taste, appearance, freshness) had the highest WTP. 40% are not WTP more for their preferred production method.
Zheng et al. 2021 [141]	Beijing, Shanghai, and Guangzhou, China	Both	Salmon	1017, primary grocery shoppers	DCE at supermarkets. Attributes: production method, product form, colour (dark vs light red), safety certification label (Y/N), price. Questions about consumers' seafood purchase patterns,	WTP is highest for the certification label > chilled > wild > dark red. Consumers' positive perception of the clean, safe, and high-quality production environment is the major drive of their high WTP

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					perceptions and attitudes towards salmon, and demographic info. 50% of respondents assigned to a consequential treatment (provided with a consequentiality script) and 50% control.	for the wild attribute. Higher-income consumers have higher utility for wild salmon.
Arru et al. 2022 [142]	Italy	FF	European seabass	318 respondents 51.7% male; >18 years	Online survey. 4 parts: brief explanation of the fish-feeding problems of aquaculture; socio-cultural characteristics of respondents; degree of respondent awareness of the consumption of insects for food purposes; questions based on prices (expensive, too expensive, cheap, too cheap) for the purchase of a sea bass fed with insect meal.	Respondents considered a price range of 6.5–13.5€ as acceptable. Consumers with great subject knowledge showed major WTP. Consumers have shown considerable price sensitivity, which does not allow the additional costs arising from the use of this sustainable alternative feed to be passed on to them.
Athnos et al. 2022 [143]	USA (North central region, NCR)	Both	Rainbow trout, yellow perch, and walleye	876 adult respondents, fish consumers at home.	Online survey, featuring screening questions, a consequentiality statement, an image illustrating the geography and states of the NCR, a DCE, and a demographics section. DCE: three labelled alternatives and a no-buy option. Attributes: species; price; form; origin; production method.	U.S. fish consumers are willing to pay premiums for NCR-sourced fish, and wild fish. Americans choose wild fish over farmed fish. Wild has the highest premium at $1.97/lb. followed by NCR-sourced at $1.64/lb.
Baldi et al. 2022 [144]	Italy	FF	General	485, below 40 years of age.	Online survey: 1, sociodemographics; 2, perceptions of farmed fish diet; 3, perceptions of eating insect-fed fish; intentions surrounding insect aquafeeds; 4, acceptance of products and intention to consume.	Perceptions of farmed fish diet were mostly on the scale midpoint. Most would eat farmed fish fed with insects if the foods were safe and fit to eat and all insect farming-related risks are controlled.
Bimbo et al. 2022 [145]	Italy	Both	General	312 Italian household that purchase fish	Best-worst method analysis. Online survey. Respondents chose the most and least important attributes when purchasing fish. Attributes: origin, price, taste, past experience, fish species, production method, storage process, sustainability certification, nutritional content.	Taste is the most relevant feature driving fish purchases. COO ranked second. Fish species and sustainability certification jointly ranked third. Negative scores found for production (less relevant).
Budhathoki et al. 2022 [146]	Denmark	Both	General	237 consumers, 18-70 years.	Web survey with 4 sections, spread through social media (ads) and malls/libraries (cookies as incentives). Info about organic fish provided at the beginning. 1) food-related lifestyle (shopping scripts, product attributes, meal preparation) – 5-point Likert scale; 2) fish eating/purchasing habits; 3) theory of planned behaviour (past experience, attitude towards organic fish, subjective norms, perceived barriers, perceived price, intention to buy) – 5-point Likert scale; 4) demographics.	Wild fish was preferred. No differences on segments regarding preferred production methods. Past experience, perceived barriers (difficulty to judge the quality) and perceived availability of organic fish were significant predictors of intention to buy organic fish. Attitudes, subjective norms, and perceived price were not significant.

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Cerveira et al. 2022 [147]	Portugal	WF	Weakfish	35 fish consumers from middle class who had tried this species	Gutted fresh fish were given to consumers to prepare in their homes. Then, consumers answered a survey: sensory evaluation (appearance, flavour and texture) of weakfish, in a 3-point scale; intentions to purchase; comparison with other commonly purchased species; aquaculture vs wild fish preference.	90% would buy weakfish because they appreciated its appearance, flavour, and texture, besides being a wild fish.
Ferfolja et al. 2022 [148]	Croatia	FF	General	303 respondents	Mixed survey (70% online, 30% in person). 1) fresh fish consumption habits; 2) subjective and objective knowledge about fresh fish from organic farming; 3) consumption of fresh organic fish; 4) perception of conventional and organic farmed fish; 5) Demographics.	39% claim medium knowledge of organic fish farming; 37% report low; 19%, very low. Most indicated that organic fresh fish is fish that has been farmed in compliance with environmental standards. Objective knowledge was low. 45% are not aware they can buy organic farmed fish at the local market. 49% are not sure if ever tried organic farmed fish. 46% are WTP for fresh organic fish. Of these, 45% are WTP 11-20% more.
Flores et al. 2022 [149]	Brazil	Both	Tambaqui, tilapia, pintado, pirarucu, salmon	1352 fish consumers	Surveys administered in person at seafood counters in supermarkets. Questions about sociodemographic attributes, fish consumption, fish knowledge and DCE. Attributes: species (tilapia and tambaqui), product form, and price.	Around 50% of consumers prefer wild fish, but around 40% does not show any preference. In general, respondents knew that tilapia is farmed, and that sardine is wild. Other species can come from both sources of production, and consumers were generally unsure about how they are sourced.
Güney et al. 2022 [150]	Greece		General	421 consumers, responsible for their household or family purchases	Survey: 1, consumption patterns; 2, attitudes towards fish; 3, sociodemographics; 4, fish consumption habits.	Taste, physical appearance, convenience, wild fish, and seller trust attitudes have a significant and positive relationship with the dependent variable (frequency of fish purchase and consumption). Fish seen as healthy and expensive. Wild fish preferred.
Ho et al. 2022 [151]	Singapore	FF	General	1011, older than 21. quotas on gender, age, and ethnicity to increase representativeness of the sample	Online survey. 5-point scales. Questions about funding, public and private, to develop feed from okara or plastic-eating larvae; if fish fed these should be labelled as so; environmental involvement; trust in food companies, regulatory bodies, news media organizations; deference to scientific authority; media attention; discussion with acquaintances about food technology; knowledge of aquaculture (T/F, with don't know	Environmental involvement was positively associated with support for funding and preference for labelling. Risk perception was negatively associated with support for funding and positively associated with preference for labelling. Benefit perception was positively associated with both. The public prefers labelling fish reared on alternative aquafeeds.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
					option); risk and benefit perception of fish fed these feeds, in terms of taste, health and environment.	
Hoerterer et al. 2022 [152]	Germany	Both	General	Attendants of a young marine researchers' conference, of AE2019, and public. Total 442.	Online or printed questionnaires. Seafood consumption; self-assessment of aquaculture knowledge; evaluation of sustainability of fish farming; seafood origin preference; attitude towards aquaculture (social, economic and environmental); definition of sustainability (only for the public); other comments; demographics.	37% have no preference of origin or depends on the product. 25% do not know origin of bought products. Median of 2 on self-assessed knowledge; Older and male respondents find aquaculture more sustainable. 52% have a positive image of aquaculture, ↑ with education, but no difference between public and science community. 61% agree that aquaculture is an economic way to produce seafood. 40% of public and 26% believe farming is not good for fish welfare, but the only way to ensure seafood availability. Other comments: aquaculture necessary for food security; pollution by antibiotics and impacts on wild; respondents are uninformed.
Hoque and Myrland 2022 [153]	Bangladesh	Both	Rui	422, responsible for family food shopping	Direct interview. DCE attributes: production method (wild, coastal farmed, inland farmed), form, type of safety inspection (national authority, local authority, no authorised safety inspection), price. Information about attributes provided. Importance of each attribute for purchase was ranked.	Consumers prefer wild > inland farmed > coast farmed. Wild-caught fish creates utility for consumers without any food safety inspection, but this is not the case for farmed fish.
Hoque et al. 2022 [154]	Bangladesh	FF	Carp	1053, responsible for food shopping.	Face-to-face survey. 1) info about consumer ambiguity and consumer confusion 2) rate their feelings on a 7-point Likert on tolerance of ambiguous situations, enjoyment in tackling problems, preference for novelty / familiarity, and preference for ambiguous situations. Confusion avoidance: questions on variety, novelty, complexity, conflict, comfort, and reliability 3) purchase intention 4) demographics.	Ambiguity tolerance positively influenced farmed fish purchase intention. Confusion avoidance is not associated with purchase intention. Income and age have a negative relationship with purchase intention. Presence of children in the family is positively associated with consumers' purchase intention.
Hoque et al. 2022 [155]	Bangladesh	Both	Rui	404, responsible for family food shopping.	Direct interview. DCE attributes: production method; origin; food safety (formalin-free vs no claim); price. Importance of each attribute for purchase ranked. Info was provided prior to DCE. Concerns about microbial contamination,	WTP for safe farmed fish was 299.98 BDT/kg (vs 220 for conventional farmed Rui). WTP for safe wild fish was 399.13 BDT/kg (vs 350 for conventional wild Rui). Consumers had a high consciousness of vitamins, fat / cholesterol, and

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
					pesticides, fat / cholesterol, preservatives, genetically modified fish, additives, and vitamins assessed on a 5-point Likert scale.	preservatives. ‘Production mode’ attribute was the first preference choice for most. WTP for imported fish < local. Consumers prefer formalin-free fish and are WTP a premium.
Hoque et al. 2022 [156]	Bangladesh	FF	General	1041, aged over 21, and responsible for buying fish and food at the household	Direct interview with structured questionnaire. Concepts of ‘consumer confusion’ and ‘consumer ambiguity’ were detailed. Respondents rated statements relating to ambiguity tolerance, confusion avoidance and fresh farmed fish knowledge (7-point Likert rating scale). Last section, demographics.	Ambiguity tolerance significantly impacted consumers’ perceived farmed fish knowledge, but confusion avoidance does not. Those with high fish consumption and who do their fish shopping personally are > likely to gather farmed fish knowledge.
Hossain et al. 2022 [157]	Bangladesh	FF	Major carp	384	Wet market survey. 1) demographics; 2) perception of farmed fish risk; 3) importance of attributes for purchase; 4) WTP for HACCP-certified carps (selecting an interval).	Premium of 24.62% more for HACCP-certified frozen major carps. Schooling, high income, consumption frequency, safety risk perception, and taste importance have a positive impact.
Kralik et al. 2022 [158]	Ohio, USA	Both	Yellow perch	Sensory evaluation: 63, consumed fish within the last 3 months, no issues with taste/smell, no allergies. Perception: 344 from Amazon Mechanical Turk.	Sensory evaluation: compared aquaponic fish with wild caught (not farmed, because wild is the highest standard). Fillets cooked at 177 °C for 10 min. Overall liking, taste liking, texture liking, and appearance liking assessed with a 9-point hedonic scale. Texture was also evaluated using a 7-point Just-About-Right scale anchored by “very much too firm” and “very much not firm enough”. Consumer perception: 6 groups – farm-raised, aquaponic or wild-caught × absence (just the name of production method) or presence (full description) of info. Scenario of purchasing fish at a grocery store; random condition assigned. Perceived tastiness and healthiness of that fish, purchasing intention, perceived info concreteness, perceived knowledge, demographics.	Aquaponic perch were comparable to wild and conventional farmed in texture, moisture content, total fat, and total protein. No differences between wild and aquaponic on sensory properties. Wild fish had the highest perceived tastiness and healthiness. Increased info ↑ perceived tastiness, healthiness and purchase intention. Info on aquaponics ↑ purchase intention to levels above wild fish.
Krešić et al. 2022 [159]	Croatia	Both	General	977, responsible for buying food, consumed fish in the last year. Nationally	The term marine aquaculture was clearly explained. 1) demographics and physical activity frequency; 2) fish consumption frequency; 3) intention to consume and beliefs about wild and farmed fish; 4) objective and subjective knowledge; 5) purchase place; 6) source of information about fish products.	4 clusters. 1) farmed fish enthusiasts (21%): mostly employed full-time; ↑ fish consumption; more interested in product info; ↑objective and subjective knowledge 2) farmed fish supporters (17%): ↑ income; 3) indifferents (44%): >younger consumers; least interested in product info. and 4) farmed fish sceptics (17%): > share of older

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				representative sample		consumers; ↓ income; mostly unemployed or retired; ↓ fish consumption; strong beliefs about wild fish; ↓ objective and subjective knowledge. Intention to consume farmed fish is related to fish consumption in general. Prejudices against farmed fish are present in all clusters: generally believe that wild fish is superior: it has a healthier diet, is healthier, better quality, more nutritious, tastes better and is firmer. Farmed fish is considered more artificial but easily available, with more guarantees and cheaper prices, less safe, but better controlled. Objective knowledge > subjective knowledge.
Masi et al. 2022 [160]	Spain, Greece, Italy, France	Both	Seabass and seabream	1500 consumers who consumed seabass and seabream over twice in the last year. Age over 18.	Online survey. 1) demographics; 2) consumption frequency and reasons for purchasing; 3) consumer approach to seabream and seabass products; 4) information and labels.	Reasons for not consuming fish: not liking smell/taste, too expensive, difficult to prepare. Quality is the most mentioned factor to affect purchase of seabass/ seabream. Wild fish preferred. Preferred info sources: unofficial over institutional sources, given by doctors and institutions. Info consumers pay attention to on labels: expiration date, catch/harvest date, origin and production method. 11 consumer profiles relevant: premium; traditional; ethical; conscious; purchasing responsible consumers, who pay little attention to labels; occasional; consumers led by value for money; convenience; woman responsible for purchase attentive to labels; modern families; healthy.
Mehar et al. 2022 [161]	Bangladesh and India	FF	Rui	One woman and one man from each household were selected. 288 respondents in Bangladesh, 270 in India. Decision makers on production,	Survey and a stated choice experiment. The choice experiment was done after the open-ended interview. Men and women of same household were interviewed separately. Respondents identified the characteristics they liked, disliked or would like improved in rui, from their perspective as a producer, guided to ensure they informed preferences relevant to the production system. Consumer perspective: because all households identified the women respondents as the primary decision makers on	In Bangladesh, pleasant taste, good appearance and larger size being the most important attributes. In India, taste and good price were the most important. For farming, poor appearance and slow growth were the strongest downsided for women in Bangladesh. Few dislikes were recorded in India. In both countries, about traits identified for improvement in production: reducing the culture period, improving fry quality, increasing disease resistance and increasing size. Size, appearance and freshness were the

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				cooking and household fish consumption.	consumption, women were asked additionally via open-ended questions what characteristics they considered when buying or cultivating fish for household consumption. Stated choice: using software 1000minds. respondents choose the relative importance of one trait over another. Traits: weight; price; length; taste; body shape; skin colour; eye colour; gill colour.	most significant for choosing farmed fish for consumption in both countries.
Peiró-Signes et al. 2022 [162]	European Union	Both	General	21627 respondents from a Eurobarometer survey	Seriousness of climate change; seafood consumption frequency and habits; wild or farmed preference; importance of attributes for purchasing; should ethical, social or environmental information be in labels; socio-demographic info. Machine learning used to assess the characteristics of the fish and aquaculture product buyers that consider environmental impact, ethical, and social information that should appear on labelling.	47% reported that sustainability info should not be in the label. Respondents considering environmental, social and ethical impact when buying fish, perceiving climate change or the deterioration of nature as the single most serious problem facing the world and those that evaluate more seriously climate change as a problem at this moment are more likely to request for sustainable labelling. EU consumers who have preference for wild products are more likely to demand sustainable labelling. Consumers who do not have any preference for wild of farmed products are less likely to demand this info to be included in the labelling.
Petereit et al. 2022 [163]	Germany (n of events = 5), Belgium (n = 1), Spain (n = 1) and Poland (n = 1).	Both	Atlantic salmon, gilthead seabream, European seabass, rainbow trout	Total of 383 respondents from 8 different knowledge transference events, under GAIN outreach activities	Interactive poster survey to identify the prevalent purchase criteria and species preferences, employing predetermined questions as well as one open-ended question. Data collected at 8 knowledge transfer events. Around 40 semi-structured interviews were conducted alongside the poster at the various knowledge transfer events.	Those who do not eat seafood say so due to vegetarian or vegan diets. Response to if certification is a purchase criterion was above 50% at all events. Most were well aware of the MSC logo, while only a few stated to ever have paid attention to ASC. Survey showed that many ignored the production method but solely focused on the certificates, as they assumed this indicated a higher sustainability. 73% stated that the price range influenced their purchasing behaviour.
Pupavac et al. 2022 [164]	Croatia	Both	General	2910, over 18 years of age	Survey: 1, fish and seafood consumption and habits; 2, sociodemographics; 3, eating habits.	COO important for around 20% of respondents. Most important sources of info: friends/family and internet. Wild fish preferred, more so for those with a moderate to high intake than those with low fish intake (68% and 52%, respectively). Those with higher fish intake mostly agreed that farmed fish are fattier than wild and of a poorer quality,

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
						while the low intake group was mostly uncertain about that. Both groups mostly were uncertain about the price of farmed fish. Most agree they do not know enough about farmed fish.
Rickard et al. 2022 [165]	California, Florida, Maine, USA	FF	Atlantic salmon	71 stakeholders: local and state government, tribal, corporate, local, journalist, non-profit, university	Semi-structured online interviews. Prior knowledge and perception of aquaculture and recirculating aquaculture systems, perception of community identity, involvement in the siting process, and attention to the siting in news and other public discourse.	Supporters believed RAS was a win-win for economic development and healthy ecosystems. Some saw investing money and attention in “artificial” land-based RAS as unfairly tipping the scales towards aquaculture and against the future of the wild fishery. Many comparisons between RAS and terrestrial agriculture. Others highlighted poor animal welfare.
Saidi et al. 2022 [166]	Italy, Spain, Tunisia and Lebanon		General	77. Over 18 years old, responsible (totally or partially) for grocery shopping. 35 from inland and 42 from seaside regions.	Focus groups: definition of fish products, context, search attributes, experience attributes, credence attributes. 2 per country.	Freshness and fish species are the most important for purchasing, which are used as proxies for quality and sensory attributes. Credence attributes such as animal welfare and sustainability received a minor attention from all the respondents. North Mediterranean (Italy and Spain) interviewees did not value production method for purchasing. However, south Mediterranean interviewees in the same study showed a preference for wild fish.
Sigurdsson et al. 2022 [167]	USA	Both	Salmon	601 for study 1 and 600 for study 2 (using Amazon Mechanical Turk crowdsourcing service).	2 studies, both designed to simulate an online grocery store, where respondent would buy fillets. DCE attributes: eco-label (study 1; MSC/ASC label; present vs absent) or sustainability tag (study 2; present vs absent); origin; production method; product form; price (conditioned to be different for farmed – cheaper – and wild).	In study 1, eco-label was the 4th (out of 5) most important attribute for consumer choice. In Study 2, sustainability tag was the 3rd most important, higher in importance than both product origin and purchase state. Consumers are WTP 23% more for fish with sustainability tags, higher than the value ascribed to the MSC label. Most were unfamiliar with frequent eco-labels.
Stancu et al. 2022 [168]	Spain, France, Germany	FF	General	500 per country. Age over 18; responsible (or partly) for the household’s food shopping; fish consumers; not professionals	Online survey. Food involvement, food innovativeness and food responsibility were used as segmentation basis. Each dimension was measured by 5 items rated on a 7-point agreement scale. Profiling factors: general attitude towards fish or fish products; category involvement with fish and fish products ; health involvement ; motives to eat aquaculture fish or fish products; domain innovativeness; perceived	1) moderate: from all countries; average values on all food lifestyle dimensions; 2) adventurous responsible: mostly from Spain and France. higher mean values on all dimensions, in particular food innovativeness and food responsibility. Mostly female; 3) conservative: mostly from Germany; low innovativeness and responsibility. Lower education. Lowest openness. Low knowledge. Low intentions to buy

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				from the fish industry. 60% female (set quota).	difficulties in preparing fish and evaluating its quality; habitual fish consumption; objective knowledge (4 items, T/F/don't know). Outcomes: intention to buy new fish products; purchase and eating frequency.	all new aquaculture products. 4) foodies: very high values on all three dimensions. Most positive attitude towards fish; high health involvement, motivation for nutrition and safety, openness, category involvement, habitual fish consumption. highest intentions to buy all new aquaculture products; 5) uninvolved: lowest on food involvement and second lowest on the other two dimensions. Least positive attitude towards fish. Lowest health involvement and motivation for nutrition. Low knowledge. Low intentions to buy all new aquaculture products.
Tran et al. 2022 [169]	Nigeria	FF	Catfish	198	Personal surveys at fish markets. Consumers had to bid for 8 products: 1) uncertified, 500 g, live; 2) uncertified, live, 1000 g; 3) certified, live, 1000 g; 4) certified, live, 500 g; 5) uncertified, smoked, 500 g; 6) certified, smoked, 500 g; 7) uncertified, smoked, 250 g; 8) certified, smoked, 250 g. Products were available for inspection. One of the products was randomly assigned to each consumer for purchasing.	Participants were generally aware of risks of consumption of unsafe fish; Consumers were WTP 3%-19% more for farmed fish certified as safe compared to uncertified.
Wongprawmas et al. 2022 [170]	Italy	Both	General	804, over 18 years old, primary food shoppers, purchasers of fish within the last 12 months	Online survey. 1) purchase habits. 2) consumer fish involvement. 3) preferences for wild fish. 4) perceived importance of sustainability. 5) ethics of fish production and consumption. 6) subjective and objective knowledge (9 statements; F, maybe F, do not know, maybe T, T). 7) misconception of the fish sector 8) attitude and purchase intent toward farmed and wild fish. 9) demographics. Two groups: control, no info provided; treatment, participants received correct info after every question in the objective knowledge section.	Median objective knowledge 6 out of 9. Subjective knowledge 4 out of 7. Participants perceived that farmed fish is cheap, nutritious, safe, and healthy. Wild fish is perceived as nutritious, healthy, and favourable. Treatment group had a more positive attitude toward farmed fish than control. Participants in the treatment group had higher intentions to purchase farmed fish than the control group.
Abaidoo et al. 2023 [171]	Great Lakes region, USA	Both	Salmon, trout, whitefish	1272, over 18 years of age, primary food shoppers in the household, who purchased	Discrete choice experiment (DCE). Attributes: species, price, origin, production method and processing form. A random half of the respondents were provided a news article describing a food fraud investigation, stating that a share of domestically consumed seafood	Apart from the price-sensitive segment, all others value national fish. Wild fish preferred to farmed for all segments and with higher WTP.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				seafood in the last year	originated internationally. Socioeconomic characteristics assessed.	
Baldi et al. 2023 [172]	Italy	FF	General	437 respondents, 55 years and older	Online survey. Prior to survey: in the information treatment group, respondents read a short text about issues related to aquafeeds and advantages of aquafeeds; in the environmental or nature priming nudge groups, respondents were shown pictures related to environmental protection activities or nature bonding moments, respectively. 1, sociodemographics; 2, perceptions of farmed fish diet; 3, perceptions of eating insect-fed fish; intentions surrounding insect aquafeeds; 4, acceptance of products intention to consume.	Informing or priming consumers with images of the environment and nature can change consumers' beliefs about alternative feeds, favouring in turn their attitude toward insect-farmed fish and their consumption intentions
Baraibar-Diez et al. 2023 [173]	Spain	FF	General	53 different stakeholders' Twitter profiles (85,186 tweets)	Sentiment analysis from tweets was performed through data mining techniques and a lexicon-based approach. Words were classified into categories such as anger, anticipation, disgust, fear, joy, negative, positive, sadness, surprise, and trust.	Aquaculture producers and associations should use this network more often to appeal to consumers. Most focus on product promotion, but they could better communicate the benefits of their activity. Negative words are often used to convey positive messages, which may hinder the effectiveness. The authors suggest homogenization of hashtags and categories to facilitate user search.
Beyer et al. 2023 [174]	Hawai, U.S.A.	Both	General	107 high school students, from 3 schools	Online survey: 1, socio-demographic data; 2, knowledge of fish production, seafood consumption and how COVID-19 impacted their fish consumption; 3, preferences for aquaculture versus wild seafood and influences on seafood purchases at home.	Higher preference for wild seafood compared to farmed in terms of taste. Farmed preferred in terms of environmental impacts. Rural residents and male students showed a significant preference for aquaculture seafood.
Bogliacino et al. 2023 [175]	Sweden (Study I and II), Denmark (I and III), Croatia (I and III), Romania (I and III), Germany (all), Czech Republic (I and	Both	Yellowfin tuna	Study I: 800 per country, age 18-74, who purchased fish in the last month. Study II: 180 per country, age 18-74, who	Study I: Participants are assigned to: mandatory information only or mandatory plus voluntary information. One label is shown accordingly for 15 s, and consumers answer 3 questions, with only one correct answer. Study II: Participants are assigned to: a time pressure treatment where the labels are shown for 10 s and a control, for 60 s. Two hypothetical purchasing scenarios. A product is described in	Mandatory information is better recalled than voluntary information. Consumers perceive and process differently labels for farmed and wild fish, relying more on quality claims for the former. Too much information on labels leads to cognitive overload and consumer confusion.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
	III), Italy (I and III), Spain (I and II) and Hungary (II).			purchased fish in the last month. Study III: 800 per country, age 18-74, who purchased fish in the last month.	text, and then respondents must complete the blank space in a label, with the most trustworthy origin claim. Study III: Hypothetical food delivery platform. Respondents are given a list of products with certain attributes to purchase from the platform. Five conditions: control (mandatory only information), control + vessel's flag state, control + port of landing, control + vessel's flag state & port of landing, information overload (same as the previous but with a non-origin related information).	
Cantillo et al. 2023 [176]	Gran Canaria, Spain	Both	Seabream and seabass	351. Adults; responsible for buying food; consumers of these species.	Online surveys. Two different survey were distributed: one with the statements written in a positive way towards aquaculture, and the other one with the same statements but written in a negative perspective towards aquaculture.	There is a higher rejection of aquaculture if the statements are presented in a negative way towards it. Variance in responses also increases.
Fong et al. 2023 [177]	California, USA	FF	General	23 leaders and experts from the industry, environmental NGOs, tribes, and academics and 57 others suggested by these	Online surveys. Basic info about respondents' history in the sector; perception of the current state of aquaculture in California; future directions for importance of the sector; taxa that should be farmed; markets for production; and how to close the gap between the amount of seafood produced and consumed.	People generally preferred California farms to focus on expanding seaweed and bivalve production, and not land-based fish or crustaceans. People showed a strong preference for local production to fill the seafood production-consumption gap in the state.
Forleo and Palmieri 2023 [178]	Italy	Both	Tuna	289, canned tuna consumers, involved in food purchasing	Online survey. 1, knowledge, awareness and concern about the environmental aspects of seafood production; 2, sustainability aspects in wild and farmed tuna; 3, purchasing choices regarding canning tuna; 4, sociodemographics.	Respondents are concerned about the environmental challenges in fish production, but over 50% has no or little knowledge of the environmental sustainability of both wild and farmed fish. Impacts for wild fish: by-catch, damage to marine ecosystems and species reproduction; for the farmed fish: feeding and risk of the virus. Most believe that wild fish has a greater environmental impact than the farmed tuna. Minimal attention is paid to environmental attributes when purchasing canned tuna, and the production method of the fish is ignored.

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
Forleo et al. 2023 [179]	Italy	Both	Tuna	251, canned tuna consumers, involved in food purchasing	Online survey. 1, knowledge and perceptions about sustainability of seafood consumption; 2, canned tuna preferences and frequency of consumption; 3, sociodemographics.	COO is the aspect most considered, while other characteristics with a higher content of environmental sustainability received minimal attention. 46% of individuals had low and 9% null personal knowledge about sustainability.
Hossain 2023 [180]	Bangladesh	Both	Aor, Boal, Rita, Hilsha, pangasius, tilapia, rui, catla	200	Personal interviews at fish purchasing moments with a semi-structured questionnaire.	Consumers' high premium (at least 61%) on wild fish is due to its taste, size, and eye appearance. However, it was found that those who preferred farmed fish do it for its freshness and bright colour while they disfavoured swollen abdomen and odour.
López-Mas et al. 2023 [181]	France, Germany, Poland, Spain, Italy	Both	General	2145 (~400 /country). including quotas for gender (50% women) and age (18-64 years). Responsible for food purchases and preparation and frequent consumers of both or at least one type of fish (wild or farmed).	Online survey: 1, beliefs: 19 items, in the format 'wild/farmed fish _____ than farmed /wild fish', 7-point Likert scale; 2, sustainability perception of farmed and wild fish. 3, sociodemographics.	Consumers perceived farmed fish as being more sustainable, locally obtained, providing environmental benefits, generating employment, improving human living conditions, generating rural development, and ensuring more fair fish prices than wild fish. Wild fish was perceived as ensuring animal welfare and being more organic but causing more environmental damage than farmed fish. Four segments: ambivalent, pro-aquaculture, pro-wild fish and impartial.
Menozzi et al. 2023 [182]	Italy	Both	General	776, over 18 of age, primary food shoppers, purchased fish in the last year.	Online survey. 1, fish consumption habits; 2, beliefs regarding wild/farmed fish with 7-point agreement scales, about safety, quality, control, availability and price; 3, subjective knowledge, same scale; 4, objective knowledge (T/F statements) with two treatment groups: control and information (correct answer and explanation after each statement; 5, attitudes towards eating fish; 6, purchase intention of wild and farmed fish; 7 demographics.	Objective and subjective knowledge affected the attitudes toward wild and farmed fish; however, only subjective knowledge had an impact on the intention to purchase wild fish. Info about production methods affected consumers' attitudes but did not directly influence their purchasing intentions.
Nguyen et al. 2023 [183]	USA	Both	Salmon and tilapia	530 from fine-dining restaurants and	Survey at fine-dining and casual restaurants. Participants indicated their agreement with statements comparing two fish entrées differing in	Half of the sample believes farmed fish is more environmentally friendly and safer to eat. Only 40% and 38% of participants indicated that farm-

Ref	Geography	Focus	Fish species	Sampling	Methods	Main results
				530 from casual. Age over 18, who had eaten at casual or fine-dining restaurants in the last 12 months	harvesting methods, country of origin, sustainability certification, and sustainability rating. Consumption habits at restaurants and sociodemographic data were collected.	raised salmon is better tasting and more nutritious for humans than wild-caught salmon, respectively. Results are similar for salmon and tilapia. USA fish is better perceived.
Olsen et al. 2023 [184]	Norway	FF	Salmon	representative of the population in gender and age distribution	Online survey: 1, demographics; 2, perceptions of salmon aquaculture industry, regarding social, ethical and environmental aspects; 3, subjective knowledge and environmental concerns. Questions used a 5-point Likert scale (1, negative to 5, positive) with “I don't know” option. The ends of each scale were labelled.	Public is generally positive toward the industry. However, respondents had more negative perceptions regarding the environmental consequences of aquaculture production and the fairness of the distribution of economic gains. Respondents were reluctant about welcoming production growth.
Rosenau et al. 2023 [185]	Germany	FF	Rainbow trout	557, Over 18 years of age, buy fish fillets occasionally.	Fillets of fish fed either a control diet or a spirulina diet (20%, replacing fishmeal) were photographed for the online DCE. Labels were digitally added to photos. Survey included questions about fish purchasing habits, familiarity with spirulina and importance of attributes for purchasing fish. Info was provided on spirulina, as FM replacement and its environmental benefits, along with info that spirulina makes the fillets yellower. DCE Attributes: fillet colour, COO, price, feed claim (none, fed with sustainable resources, fed without FM, fed on plant-based proteins, fed with spirulina).	Respondents had the strongest preferences for fillets of domestic origin ahead of Denmark and Turkey. Feed claims were not significant, except for “fed with sustainable resources”, which had marginal impact. Change in colour does not prevent consumers from purchasing the fillets.
Zheng et al. 2023 [186]	USA	Both	Salmon	2209, Age over 18, consumers of seafood	DCE with 1 lb fillets. Attributes: production method (non-genetically modified farmed, genetically modified farmed, wild), product form and price. Survey also included questions about perceived safety, sustainability and welfare of wild and farmed fish	Wild salmon preferred with a higher WTP. WTP is significantly less for GM salmon than non-GM farmed or wild-caught salmon.

References

1. Peavey S, Work T, Riley J. Consumer attitudes toward fresh and frozen fish. *J Aquat Food Prod Technol*. 1994;3(2):71-87. doi:10.1300/J030v03n02_07
2. Sylvia G, Morrissey MT, Graham T, Garcia S. Organoleptic qualities of farmed and wild salmon. *J Aquat Food Prod Technol*. 1995;4(1):51-64. doi:10.1300/J030v04n01_04
3. Arvanitoyannis IS, Krystallis A, Panagiotaki P, Theodorou AJ. A marketing survey on Greek consumers' attitudes towards fish. *Aquac Int*. 2004;12(3):259-279. doi:10.1023/B:AQUI.0000036137.29397.12
4. Frewer LJ, Kole A, van de Kroon SM, de Lauwere C. Consumer attitudes towards the development of animal-friendly husbandry systems. *J Agric Environ Ethics*. 2005;18(4):345-367. doi:10.1007/s10806-005-1489-2
5. Longstaff HA. Frankenfish, monsatan, and killer canola making good decisions in the midst of environmental controversy. *J Environ Inform*. 2005;6(1):33-45. doi:10.3808/jei.200500053
6. Budak F, Budak DB, Kacira OO, Yavuz MC. Consumer willingness to pay for organic sea bass in Turkey. *Isr J Aquac*. 2006;58(2):116-123. doi:10.46989/001c.20431
7. Drake SL, Drake MA, Daniels HV, Yates MD. Sensory properties of wild and aquacultured southern flounder (*Paralichthys lethostigma*). *J Sens Stud*. 2006;21(2):218-227. doi:10.1111/j.1745-459X.2006.00062.x
8. Verbeke W, Sioen I, Brunsø K, Henauw S, Camp J. Consumer perception versus scientific evidence of farmed and wild fish: Exploratory insights from Belgium. *Aquac Int*. 2007;15(2):121-136. doi:10.1007/s10499-007-9072-7
9. Verbeke W, Vanhonacker F, Sioen I, Van Camp J, De Henauw S. Perceived importance of sustainability and ethics related to fish: A consumer behavior perspective. *Ambio*. 2007;36(7):580-585. doi:10.1579/0044-7447(2007)36[580:Piosae]2.0.Co;2
10. Li D, Ramaswamy S, Tudor KW, Spaulding AD. The North Central aquaculture region as a potential market for farm-raised hybrid striped bass. *Aquac Econ Manag*. 2008;12(2):107-129. doi:10.1080/13657300802110752
11. Quagrainie K, Hart S, Brown P. Consumer acceptance of locally grown food: The case of Indiana aquaculture products. *Aquac Econ Manag*. 2008;12(1):54-70. doi:10.1080/13657300801962591

12. Gaviglio A, Demartini E. Consumer attitudes towards farm-raised and wild-caught fish: variables of product perception. *New Medit*. 2009;8(3):34-40.
13. Honkanen P, Olsen SO. Environmental and animal welfare issues in food choice: The case of farmed fish. *Br Food J*. 2009;111(2-3):293-309. doi:10.1108/00070700910941480
14. Kole APW, Altintzoglou T, Schelvis-Smit RAAM, Luten JB. The effects of different types of product information on the consumer product evaluation for fresh cod in real life settings. *Food Qual Prefer*. 2009;20(3):187-194. doi:10.1016/j.foodqual.2008.09.003
15. Altintzoglou T, Verbeke W, Vanhonacker F, Luten J. The image of fish from aquaculture among Europeans: Impact of exposure to balanced information. *J Aquat Food Prod Technol*. 2010;19(2):103-119. doi:10.1080/10498850.2010.492093
16. Amberg SM, Hall TE. Precision and rhetoric in media reporting about contamination in farmed salmon. *Sci Commun*. 2010;32(4):489-513. doi:10.1177/1075547009357599
17. Ariji M. Conjoint analysis of consumer preference for bluefin tuna. *Fish Sci*. 2010;76(6):1023-1028. doi:10.1007/s12562-010-0297-4
18. Fernández-Polanco J, Luna L. Analysis of perceptions of quality of wild and cultured seabream in Spain. *Aquac Econ Manag*. 2010;14(1):43-62. doi:10.1080/13657300903566878
19. Myers JJ, Govindasamy R, Ewart JW, et al. Consumer analysis in ethnic live seafood markets in the northeast region of the United States. *J Food Prod Mark*. 2010;16(2):147-165. doi:10.1080/10454440903415477
20. Olesen I, Alfnes F, Røra MB, Kolstad K. Eliciting consumers' willingness to pay for organic and welfare-labelled salmon in a non-hypothetical choice experiment. *Livest Sci*. 2010;127(2-3):218-226. doi:10.1016/j.livsci.2009.10.001
21. Young JA, Little DC, Watterson A, et al. Growing Green: The emergent role of Non-Tilapia attributes in marketing Tilapia. *Aquac Econ Manag*. 2010;14(1):63-79. doi:10.1080/13657300903566886
22. Altintzoglou T, Vanhonacker F, Verbeke W, Luten J. Association of health involvement and attitudes towards eating fish on farmed and wild fish consumption in Belgium, Norway and Spain. *Aquac Int*. 2011;19(3):475-488. doi:10.1007/s10499-010-9363-2
23. Hansen H, Onozaka Y. When diseases hit aquaculture: An experimental study of spillover effects from negative publicity. *Mar Resour Econ*. 2011;26(4):281-291. doi:10.5950/0738-1360-26.4.281

24. Pohar J. Detection and comparison of the sensory quality of wild and farmed brown trout (*Salmo trutta*) by consumers. *Acta Agricult Slov*. 2011;98(1):45-50. doi:10.14720/aas.2011.98.1.14596
25. Rudd MA, Pelletier N, Tyedmers P. Preferences for health and environmental attributes of farmed salmon amongst southern ontario salmon consumers. *Aquac Econ Manag*. 2011;15(1):18-45. doi:10.1080/13657305.2011.549405
26. Solgaard HS, Yang Y. Consumers' perception of farmed fish and willingness to pay for fish welfare. *Br Food J*. 2011;113(8):997-1010. doi:10.1108/00070701111153751
27. Vanhonacker F, Altintzoglou T, Luten J, Verbeke W. Does fish origin matter to European consumers?: Insights from a consumer survey in Belgium, Norway and Spain. *Br Food J*. 2011;113(4):535-549. doi:10.1108/00070701111124005
28. Whitmarsh D, Palmieri MG. Consumer behaviour and environmental preferences: A case study of Scottish salmon aquaculture. *Aquac Res*. 2011;42(SUPPL. 1):142-147. doi:10.1111/j.1365-2109.2010.02672.x
29. Claret A, Guerrero L, Aguirre E, et al. Consumer preferences for sea fish using conjoint analysis: Exploratory study of the importance of country of origin, obtaining method, storage conditions and purchasing price. *Food Qual Prefer*. 2012;26(2):259-266. doi:10.1016/j.foodqual.2012.05.006
30. Davidson K, Pan ML, Hu WY, Poerwanto D. Consumers' willingness to pay for aquaculture fish products vs. wild-caught seafood - a case study in Hawaii. *Aquac Econ Manag*. 2012;16(2):136-154. doi:10.1080/13657305.2012.678554
31. de Moura AP, Cunha LM, Castro-Cunha M, Lima RC. A comparative evaluation of women's perceptions and importance of sustainability in fish consumption: An exploratory study among light consumers with different education levels. *Manag Environ Qual*. 2012;23(4):451-461. doi:10.1108/14777831211232263
32. Freeman S, Vigoda-Gadot E, Sterr H, et al. Public attitudes towards marine aquaculture: A comparative analysis of Germany and Israel. *Environ Sci Policy*. 2012;22:60-72. doi:10.1016/j.envsci.2012.05.004
33. Lawley M, Birch D, Hamblin D. An exploratory study into the role and interplay of intrinsic and extrinsic cues in Australian consumers' evaluations of fish. *Australas Mark J*. 2012;20(4):260-267. doi:10.1016/j.ausmj.2012.05.014
34. Roheim CA, Sudhakaran PO, Durham CA. Certification of shrimp and salmon for best aquaculture practices: Assessing consumer preferences in Rhode Island. *Aquac Econ Manag*. 2012;16(3):266-286. doi:10.1080/13657305.2012.713075
35. Stefani G, Scarpa R, Cavicchi A. Exploring consumer's preferences for farmed sea bream. *Aquac Int*. 2012;20(4):673-691. doi:10.1007/s10499-011-9495-z

36. Cardoso C, Lourenço H, Costa S, Gonçalves S, Nunes ML. Survey into the seafood consumption preferences and patterns in the portuguese population. Gender and regional variability. *Appetite*. 2013;64:20-31. doi:10.1016/j.appet.2012.12.022
37. Fernández-Polanco J, Loose SM, Luna L. Are retailers' preferences for seafood attributes predictive for consumer wants? Results from a choice experiment for seabream (*Sparus aurata*). *Aquac Econ Manag*. 2013;17(2):103-122. doi:10.1080/13657305.2013.772262
38. Grimsrud KM, Nielsen HM, Navrud S, Olesen I. Households' willingness-to-pay for improved fish welfare in breeding programs for farmed Atlantic salmon. *Aquaculture*. 2013;372:19-27. doi:10.1016/j.aquaculture.2012.10.009
39. Hall TE, Amberg SM. Factors influencing consumption of farmed seafood products in the Pacific northwest. *Appetite*. 2013;66:1-9. doi:10.1016/j.appet.2013.02.012
40. Kalshoven K, Meijboom FLB. Sustainability at the crossroads of fish consumption and production ethical dilemmas of fish buyers at retail organizations in The Netherlands. *J Agric Environ Ethics*. 2013;26(1):101-117. doi:10.1007/s10806-011-9367-6
41. Kupsala S, Jokinen P, Vinnari M. Who cares about farmed fish? Citizen perceptions of the welfare and the mental abilities of fish. *J Agric Environ Ethics*. 2013;26(1):119-135. doi:10.1007/s10806-011-9369-4
42. Mauracher C, Tempesta T, Vecchiato D. Consumer preferences regarding the introduction of new organic products. The case of the Mediterranean sea bass (*Dicentrarchus labrax*) in Italy. *Appetite*. 2013;63:84-91. doi:10.1016/j.appet.2012.12.009
43. Pieniak Z, Vanhonacker F, Verbeke W. Consumer knowledge and use of information about fish and aquaculture. *Food Policy*. 2013;40:25-30. doi:10.1016/j.foodpol.2013.01.005
44. Rodríguez GR, Bande R, Villasante S. Origins matter: (no) market integration between cultured and wild gilthead sea bream in the spanish seafood market. *Aquac Econ Manag*. 2013;17(4):380-397. doi:10.1080/13657305.2013.825932
45. Schlag AK, Ystgaard K. Europeans and aquaculture: Perceived differences between wild and farmed fish. *Br Food J*. 2013;115(2):209-222. doi:10.1108/00070701311302195
46. Vanhonacker F, Pieniak Z, Verbeke W. European consumer image of farmed fish, wild fish, seabass and seabream. *Aquac Int*. 2013;21(5):1017-1033. doi:10.1007/s10499-012-9609-2

47. Claret A, Guerrero L, Ginés R, et al. Consumer beliefs regarding farmed versus wild fish. *Appetite*. 2014;79:25-31. doi:10.1016/j.appet.2014.03.031
48. Guy J, Nottingham S. Fillet yield, biochemical composition, and consumer acceptance of farmed and wild mulloway. *J Aquat Food Prod Technol*. 2014;23(6):608-620. doi:10.1080/10498850.2012.750636
49. Chen X, Alfnes F, Rickertsen K. Consumer preferences, ecolabels, and effects of negative environmental information. *AgBioForum*. 2015;18(3):327-336.
50. Ellingsen K, Grimsrud K, Nielsen HM, et al. Who cares about fish welfare? A Norwegian study. *Br Food J*. 2015;117(1):257-273. doi:10.1108/bfj-08-2013-0223
51. Feucht Y, Zander K. Of earth ponds, flow-through and closed recirculation systems - German consumers' understanding of sustainable aquaculture and its communication. *Aquaculture*. 2015;438:151-158. doi:10.1016/j.aquaculture.2015.01.005
52. Koral S, Tufan B, Başçınar N, Köse S. Quality changes and shelf-life of cultured and wild hot-smoked mediterranean horse mackerel (*Trachurus mediterraneus*, Steindachner, 1868) at frozen storage (-20±2°C). *Turk J Fish Aquat Sci*. 2015;15(2):495-504. doi:10.4194/1303-2712-v15_2_37
53. Polymeros K, Kaimakoudi E, Schinaraki M, Batzios C. Analysing consumers' perceived differences in wild and farmed fish. *Br Food J*. 2015;117(3):1007-1016. doi:10.1108/BFJ-12-2013-0362
54. Alexander KA, Freeman S, Potts T. Navigating uncertain waters: European public perceptions of integrated multi trophic aquaculture (IMTA). *Environ Sci Policy*. 2016;61:230-237. doi:10.1016/j.envsci.2016.04.020
55. Alexander KA, Angel D, Freeman S, et al. Improving sustainability of aquaculture in Europe: Stakeholder dialogues on Integrated Multi-trophic Aquaculture (IMTA). *Environ Sci Policy*. 2016;55:96-106. doi:10.1016/j.envsci.2015.09.006
56. Ankamah-Yeboah I, Nielsen M, Nielsen R. Price premium of organic salmon in Danish retail sale. *Ecol Econ*. 2016;122:54-60. doi:10.1016/j.ecolecon.2015.11.028
57. Banovic M, Krystallis A, Guerrero L, Reinders MJ. Consumers as co-creators of new product ideas: An application of projective and creative research techniques. *Food Res Int*. 2016;87:211-223. doi:10.1016/j.foodres.2016.07.010
58. Bronnmann J, Asche F. The value of product attributes, brands and private labels: An analysis of frozen seafood in Germany. *J Agric Econ*. 2016;67(1):231-244. doi:10.1111/1477-9552.12138
59. Bronnmann J, Loy JP, Schroeder KJ. Characteristics of demand structure and preferences for wild and farmed seafood in Germany: An application of QUAIDS

modeling with correction for sample selection. *Mar Resour Econ*. 2016;31(3):281-300. doi:10.1086/686692

60. Claret A, Guerrero L, Gartzia I, Garcia-Quiroga M, Ginés R. Does information affect consumer liking of farmed and wild fish? *Aquaculture*. 2016;454:157-162. doi:10.1016/j.aquaculture.2015.12.024
61. Darko FA, Quagrainie KK, Chenyambuga S. Consumer preferences for farmed tilapia in Tanzania: A choice experiment analysis. *J Appl Aquac*. 2016;28(3):131-143. doi:10.1080/10454438.2016.1169965
62. Jonell M, Crona B, Brown K, Rönnbäck P, Troell M. Eco-labeled seafood: Determinants for (blue) green consumption. *Sustainability*. 2016;8(9)doi:10.3390/su8090884
63. Mancuso T, Baldi L, Gasco L. An empirical study on consumer acceptance of farmed fish fed on insect meals: the Italian case. *Aquac Int*. 2016;24(5):1489-1507. doi:10.1007/s10499-016-0007-z
64. Reinders MJ, Banović M, Guerrero L, Krystallis A. Consumer perceptions of farmed fish: A cross-national segmentation in five European countries. *Br Food J*. 2016;118(10):2581-2597. doi:10.1108/BFJ-03-2016-0097
65. Zhou GZ, Hu WY, Huang WC. Are consumers willing to pay more for sustainable products? A study of eco-labeled tuna steak. *Sustainability*. 2016;8(5):494. doi:10.3390/su8050494
66. Bronnmann J, Asche F. Sustainable seafood from aquaculture and wild fisheries: insights from a discrete choice experiment in Germany. *Ecol Econ*. 2017;142:113-119. doi:10.1016/j.ecolecon.2017.06.005
67. Froehlich HE, Gentry RR, Rust MB, Grimm D, Halpern BS. Public perceptions of aquaculture: Evaluating spatiotemporal patterns of sentiment around the world. *PLoS One*. 2017;12(1):e0169281. doi:10.1371/journal.pone.0169281
68. Kim G, Duffy LN, Jodice LW, Norman WC. Coastal tourist interest in value-ddded, aquaculture-based, culinary tourism opportunities. *Coast Manag*. 2017;45(4):310-329. doi:10.1080/08920753.2017.1327345
69. Milicic V, Thorarinsdottir R, Dos Santos M, Hancic MT. Commercial aquaponics approaching the European market: To consumers' perceptions of aquaponics products in Europe. *Water*. 2017;9(2):80. doi:10.3390/w9020080
70. Murray G, Wolff K, Patterson M. Why eat fish? Factors influencing seafood consumer choices in British Columbia, Canada. *Ocean Coast Manag*. 2017;144:16-22. doi:10.1016/j.ocecoaman.2017.04.007

71. Pereira LN, Mendes JC, Mendes JP. A marketing strategy for a new functional fish. *Aquac Econ Manag*. 2017;21(3):334-354. doi:10.1080/13657305.2016.1185197
72. Popoff M, MacLeod M, Leschen W. Attitudes towards the use of insect-derived materials in Scottish salmon feeds. *J Insects Food Feed*. 2017;3(2):131-138. doi:10.3920/JIFF2016.0032
73. Regnier E, Bayramoglu B. Competition between farmed and wild fish: The French sea bass and sea bream markets. *Aquac Econ Manag*. 2017;21(3):355-375. doi:10.1080/13657305.2016.1189012
74. Rickertsen K, Alfnes F, Combris P, Enderli G, Issanchou S, Shogren JF. French consumers' attitudes and preferences toward wild and farmed fish. *Mar Resour Econ*. 2017;32(1):59-81. doi:10.1086/689202
75. Risius A, Janssen M, Hamm U. Consumer preferences for sustainable aquaculture products: Evidence from in-depth interviews, think aloud protocols and choice experiments. *Appetite*. 2017;113:246-254. doi:10.1016/j.appet.2017.02.021
76. Short G, Yue C, Anderson N, Russell C, Phelps N. Consumer perceptions of aquaponic systems. *HortTechnology*. 2017;27(3):358-366. doi:10.21273/HORTTECH03606-16
77. Tomić M, Lucević Z, Tomljanović T, Matulić D. Wild-caught versus farmed fish – consumer perception. *Ribar Croat J Fish*. 2017;75(2):41-50. doi:10.1515/cjf-2017-0007
78. Uchida H, Roheim CA, Johnston RJ. Balancing the health risks and benefits of seafood: How does available guidance affect consumer choices? . *Am J Agric Econ*. 2017;99(4):1056-1077. doi:10.1093/ajae/aax025
79. van Osch S, Hynes S, O'Higgins T, Hanley N, Campbell D, Freeman S. Estimating the Irish public's willingness to pay for more sustainable salmon produced by integrated multi-trophic aquaculture. *Mar Policy*. 2017;84:220-227. doi:10.1016/j.marpol.2017.07.005
80. Yip W, Knowler D, Haider W, Trenholm R. Valuing the willingness-to-pay for sustainable seafood: integrated multitrophic versus closed containment aquaculture. *Can J Agric Econ*. 2017;65(1):93-117. doi:10.1111/cjag.12102
81. Ankamah-Yeboah I, Jacobsen JB, Olsen SB. Innovating out of the fishmeal trap: The role of insect-based fish feed in consumers’ preferences for fish attributes. *Br Food J*. 2018;120(10):2395-2410. doi:10.1108/BFJ-11-2017-0604
82. Antão-Geraldes AM, Hungulo SR, Pereira E, Teixeira A, Teixeira A, Rodrigues S. Body composition and sensory quality of wild and farmed brown-trout (*Salmo*

trutta) and of farmed rainbow-trout (*Oncorhynchus mykiss*). *Cienc Rural*. 2018;48(9):190. doi:10.1590/0103-8478cr20180190
83. Bronnmann J, Hoffmann J. Consumer preferences for farmed and ecolabeled turbot: A North German perspective. *Aquac Econ Manag*. 2018;22(3):342-361. doi:10.1080/13657305.2018.1398788
84. Genschick S, Marinda P, Tembo G, Kaminski AM, Thilsted SH. Fish consumption in urban Lusaka: The need for aquaculture to improve targeting of the poor. *Aquaculture*. 2018;492:280-289. doi:10.1016/j.aquaculture.2018.03.052
85. Hinkes C, Schulze-Ehlers B. Consumer attitudes and preferences towards pangasius and tilapia: The role of sustainability certification and the country of origin. *Appetite*. 2018;127:171-181. doi:10.1016/j.appet.2018.05.001
86. Weitzman J, Bailey M. Perceptions of aquaculture ecolabels: A multi-stakeholder approach in Nova Scotia, Canada. *Mar Policy*. 2018;87:12-22. doi:10.1016/j.marpol.2017.09.037
87. Zander K, Feucht Y. Consumers' willingness to pay for sustainable seafood made in Europe. *J Int Food Agribus Mark*. 2018;30(3):251-275. doi:10.1080/08974438.2017.1413611
88. Zander K, Risius A, Feucht Y, Janssen M, Hamm U. Sustainable aquaculture products: Implications of consumer awareness and of consumer preferences for promising market communication in Germany. *J Aquat Food Prod Technol*. 2018;27(1):5-20. doi:10.1080/10498850.2017.1390028
89. Ankamah-Yeboah I, Jacobsen JB, Olsen SB, Nielsen M, Nielsen R. The impact of animal welfare and environmental information on the choice of organic fish: An empirical investigation of German trout consumers. *Mar Resour Econ*. 2019;34(3):248-266. doi:10.1086/705235
90. Banovic M, Reinders MJ, Claret A, Guerrero L, Krystallis A. A cross-cultural perspective on impact of health and nutrition claims, country-of-origin and eco-label on consumer choice of new aquaculture products. *Food Res Int*. 2019;123:36-47. doi:10.1016/j.foodres.2019.04.031
91. Banovic M, Reinders MJ, Claret A, Guerrero L, Krystallis A. "One Fish, Two Fish, Red Fish, Blue Fish": How ethical beliefs influence consumer perceptions of "blue" aquaculture products? *Food Qual Prefer*. 2019;77:147-158. doi:10.1016/j.foodqual.2019.05.013
92. Ferrer Llagostera P, Kallas Z, Reig L, Amores de Gea D. The use of insect meal as a sustainable feeding alternative in aquaculture: Current situation, Spanish consumers' perceptions and willingness to pay. *J Clean Prod*. 2019;229:10-21. doi:10.1016/j.jclepro.2019.05.012

93. Flaherty M, Reid G, Chopin T, Latham E. Public attitudes towards marine aquaculture in Canada: insights from the Pacific and Atlantic coasts. *Aquac Int*. 2019;27(1):9-32. doi:10.1007/s10499-018-0312-9
94. Greenfeld A, Becker N, Bornman JF, dos Santos MJ, Angel D. Consumer preferences for aquaponics: A comparative analysis of Australia and Israel. *J Environ Manag*. 2020;257:10.1016/j.jenvman.2019.109979. doi:10.1016/j.jenvman.2019.109979
95. Guney OI. Consumers' perceived differences between wild and farmed fish: A survey study in Turkey. *J Aquat Food Prod Technol*. 2019;28(2):210-220. doi:10.1080/10498850.2019.1572684
96. Hilger J, Hallstein E, Stevens AW, Villas-Boas SB. Measuring willingness to pay for environmental attributes in seafood. *Environ Resour Econ*. 2019;73(1):307-332. doi:10.1007/s10640-018-0264-6
97. Hynes S, Ravagnan E, Gjerstad B. Do concerns for the environmental credentials of salmon aquaculture translate into WTP a price premium for sustainably farmed fish? A contingent valuation study in Ireland and Norway. *Aquac Int*. 2019;27(6):1709-1723. doi:10.1007/s10499-019-00425-y
98. Kulesz MM, Lundh T, de Koning DJ, Lagerkvist CJ. Dissuasive effect, information provision, and consumer reactions to the term ‘Biotechnology’: The case of reproductive interventions in farmed fish. *PLoS One*. 2019;14(9):e0222494. doi:10.1371/journal.pone.0222494
99. Lee MK, Nam J. The determinants of live fish consumption frequency in South Korea. *Food Res Int*. 2019;120:382-388. doi:10.1016/j.foodres.2019.03.005
100. Quagrainie KK. Consumer willingness to pay for a saline fish species grown in the US midwest: The case of striped bass, *Morone saxatilis*. *J World Aquac Soc*. 2019;50(1):163-171. doi:10.1111/jwas.12464
101. Ramalho Ribeiro A, Altintzoglou T, Mendes J, Nunes ML, Dinis MT, Dias J. Farmed fish as a functional food: Perception of fish fortification and the influence of origin – Insights from Portugal. *Aquaculture*. 2019;501:22-31. doi:10.1016/j.aquaculture.2018.11.002
102. Reig L, Escobar C, Carrassón M, et al. Aquaculture perceptions in the Barcelona metropolitan area from fish and seafood wholesalers, fishmongers, and consumers. *Aquaculture*. 2019;510:256-266. doi:10.1016/j.aquaculture.2019.05.066
103. Richard NL, Pivarnik LF. Rhode Island branding program for local seafood: Consumer perceptions, awareness, and willingness-to-pay. *J Agric Food Syst Community Dev*. 2019;9(2):13-29. doi:10.5304/jafscd.2020.092.011

104. Risius A, Hamm U, Janssen M. Target groups for fish from aquaculture: Consumer segmentation based on sustainability attributes and country of origin. *Aquaculture*. 2019;499:341-347. doi:10.1016/j.aquaculture.2018.09.044

105. van Osch S, Hynes S, Freeman S, O'Higgins T. Estimating the public's preferences for sustainable aquaculture: A country comparison. *Sustainability*. 2019;11(3):569. doi:10.3390/su11030569

106. Yeşilsu AF, Özyurt G, Dağtekin M, Alp-Erbay E. Wild fish vs. Farmed fish: Consumer perception in Turkey. *Aquac Stud*. 2019;19(1):37-43. doi:10.4194/2618-6381-v19_1_04

107. Yi S. Contingent valuation of sustainable integrated agriculture-aquaculture products: The case of rice-fish farming systems in South Korea. *Agronomy*. 2019;9(10):601. doi:10.3390/agronomy9100601

108. Yi S. Determinants of consumers' purchasing behavior for certified aquaculture products in South Korea. *Sustainability*. 2019;11(14):3840. doi:10.3390/su11143840

109. Abdikoglu DI, Azabagaoglu MO, Unakitan G. An econometric analysis of factors affecting fish consumption: The case of Tekirdag, Turkey. *KSU J Agric Nat*. 2020;23(2):446-452. doi:10.18016/ksutarimdoga.vi.623343

110. Alam MA, Alfnes F. Consumer preferences for fish attributes in Bangladesh: A choice experiment. *J Int Food Agribus Mark*. 2020;32(5):425-440. doi:10.1080/08974438.2019.1697409

111. Antão-Geraldes AM, Hungulo SR, Pereira E, Teixeira A, Teixeira A, Rodrigues S. Sensory quality and consumers' acceptance towards wild and farmed brown-trout (*Salmo trutta*) and farmed rainbow-trout (*Oncorhynchus mykiss*). *Braz J Food Tech*. 2020;23:10419. doi:10.1590/1981-6723.10419

112. Chikudza L, Gauzente C, Guillotreau P, Alexander KA. Producer perceptions of the incentives and challenges of adopting ecolabels in the European finfish aquaculture industry: A Q-methodology approach. *Mar Policy*. 2020;121:104176. doi:10.1016/j.marpol.2020.104176

113. Garlock T, Nguyen L, Anderson J, Musumba M. Market potential for Gulf of Mexico farm-raised finfish. *Aquac Econ Manag*. 2020;24(2):128-142. doi:10.1080/13657305.2019.1691676

114. Hartmann C, Siegrist M. Our daily meat: Justification, moral evaluation and willingness to substitute. *Food Qual Prefer*. 2020;80:103799. doi:10.1016/j.foodqual.2019.103799

115. Hoque MZ, Alam MN. Consumers' knowledge discrepancy and confusion in intent to purchase farmed fish. *Br Food J*. 2020;122(11):3567-3583. doi:10.1108/bfj-01-2019-0021
116. Kitano S, Yamamoto N. The role of consumer knowledge, experience,and heterogeneity in fish consumption: Policy lessons from Japan. *J Retail Consum Serv*. 2020;56:102151. doi:10.1016/j.jretconser.2020.102151
117. Menozzi D, Nguyen TT, Sogari G, et al. Consumers' preferences and willingness to pay for fish products with health and environmental labels: Evidence from five European countries. *Nutrients*. 2020;12(9):2650. doi:10.3390/nu12092650
118. Pulcini D, Franceschini S, Buttazzoni L, Giannetti C, Capoccioni F. Consumer preferences for farmed seafood: An Italian case study. *J Aquat Food Prod Technol*. 2020;29(5):445-460. doi:10.1080/10498850.2020.1749201
119. Ruiz-Chico J, Biedma-Ferrer JM, Pea-Snchez AR, Jimnez-Garca M. Acceptance of aquaculture as compared with traditional fishing in the province of Cadiz (Spain): an empirical study from the standpoint of social carrying capacity. *Rev Aquac*. 2020;12(4):2429-2445. doi:10.1111/raq.12442
120. Ruiz-Chico J, Biedma-Ferrer JM, Peña-Sánchez AR, Jiménez-García M. Social acceptance of aquaculture in Spain: An instrument to achieve sustainability for society. *J Environ Res Public Health*. 2020;17(18):1-12. doi:10.3390/ijerph17186628
121. Ruiz-Chico J, Peña-Sánchez AR, Biedma-Ferrer JM, Jiménez-García M. Social acceptance of aquaculture in andalusian atlantic coast (Spain): An emerging economy sector. *Foods*. 2020;9(7):910. doi:10.3390/foods9070910
122. Yang Y, Hobbs JE, Natcher DC. Assessing consumer willingness to pay for Arctic food products. *Food Policy*. 2020;92:101846. doi:10.1016/j.foodpol.2020.101846
123. Allegro R, Calagna A, Lo Monaco D, et al. The assessment of the attitude of Sicilian consumers towards wild and farmed seafood products – a sample survey. *Br Food J*. 2021;123(7):2506-2536. doi:10.1108/BFJ-09-2020-0853
124. Asche F, Bronnmann J, Cojocaru AL. The value of responsibly farmed fish: A hedonic price study of ASC-certified whitefish. *Ecol Econ*. 2021;188:107135. doi:10.1016/j.ecolecon.2021.107135
125. Bazoche P, Poret S. Acceptability of insects in animal feed: A survey of French consumers. *J Consum Behav*. 2021;20(2):251-270. doi:10.1002/cb.1845
126. Budhathoki M, Zolner A, Nielsen T, Reinbach HC. The role of production method information on sensory perception of smoked salmon – A mixed-method study from Denmark. *Food Qual Prefer*. 2021;94:104325. doi:10.1016/j.foodqual.2021.104325

127. Cantillo J, Martin JC, Roman C. A best-worst measure of attitudes toward buying seabream and seabass products: An application to the island of Gran Canaria. *Foods*. 2021;10(1):90. doi:10.3390/foods10010090
128. Carrassón M, Soler-Membrives A, Constenla M, et al. Information impact on consumers' perceptions towards aquaculture: Dismantling the myth about feeds for farmed fish. *Aquaculture*. 2021;544:737137. doi:10.1016/j.aquaculture.2021.737137
129. Hoque MZ. Sustainability indicators for sustainably-farmed fish in Bangladesh. *Sustain Prod Consum*. 2021;27:115-127. doi:10.1016/j.spc.2020.10.020
130. Loncaric D, Dujmic E, Kresic G. Assessment of the intention to consume farmed fish using the theory of planned behaviour - Scale validation. *J Polytech Rijeka*. 2021;9(1):1-17. doi:10.31784/zvr.9.1.1
131. López-Mas L, Claret A, Reinders MJ, Banovic M, Krystallis A, Guerrero L. Farmed or wild fish? Segmenting European consumers based on their beliefs. *Aquaculture*. 2021;532:735992. doi:10.1016/j.aquaculture.2020.735992
132. Malcorps W, Newton RW, Maiolo S, et al. Global Seafood Trade: Insights in Sustainability Messaging and Claims of the Major Producing and Consuming Regions. *Sustainability*. 2021;13(21)doi:10.3390/su132111720
133. Meng T, Wang C, Florkowski WJ, Yang Z. Determinants of urban consumer expenditure on aquatic products in Shanghai, China. *Aquac Econ Manag*. 2021;27(1):1-24. doi:10.1080/13657305.2021.1996480
134. Mitra S, Khatun MN, Prodhan MMH, Khan MA. Consumer preference, willingness to pay, and market price of capture and culture fish: Do their attributes matter? *Aquaculture*. 2021;544:737139. doi:10.1016/j.aquaculture.2021.737139
135. Runge KK, Shaw BR, Witzling L, Hartleb C, Yang S, Peroff DM. Social license and consumer perceptions of farm-raised fish. *Aquaculture*. 2021;530:735920. doi:10.1016/j.aquaculture.2020.735920
136. Sagun OK, Sayğı H. Consumption of fishery products in Turkey's coastal regions. *Br Food J*. 2021;123(9):3070-3084. doi:10.1108/BFJ-05-2020-0442
137. Sajiki T, Lu YH. Japanese consumer preference for raw Fish: best-worst scaling method. *J Mar Sci Tech*. 2021;29(6):810-818. doi:10.51400/2709-6998.2560
138. Suarez-Caceres GP, Fernandez-Cabanas VM, Lobillo-Eguibar J, Perez-Urrestarazu L. Consumers' knowledge, attitudes and willingness to pay for aquaponic products in Spain and Latin America. *Int J Gastron Food Sci*. 2021;24:100350. doi:10.1016/j.ijgfs.2021.100350

139. Tezzo X, Aung HM, Belton B, Oosterveer P, Bush SR. Consumption practices in transition: Rural-urban migration and the food fish system in Myanmar. *Geoforum*. 2021;127:33-45. doi:10.1016/j.geoforum.2021.09.013
140. Witter A, Murray G, Sumaila UR. Consumer seafood preferences related to alternative food networks and their value chains. *Mar Policy*. 2021;131:104694. doi:10.1016/j.marpol.2021.104694
141. Zheng QJ, Wang HH, Shogren JF. Fishing or aquaculture? Chinese consumers' stated preference for the growing environment of salmon through a choice experiment and the consequentiality effect. *Mar Resour Econ*. 2021;36(1):23-42. doi:10.1086/711385
142. Arru B, Furesi R, Pulina P, Madau FA. Price sensitivity of fish fed with insect meal: An analysis on Italian consumers. *Sustainability*. 2022;14(11):6657. doi:10.3390/su14116657
143. Athnos A, de Souza SV, Quagrainie K, et al. Are U.S. consumers wilting to pay more by the take? An analysis of preferences for Great Lakes region fish. *Agric Resour Econ Rev*. 2022:1-26. doi:10.1017/age.2022.18
144. Baldi L, Mancuso T, Peri M, Gasco L, Trentinaglia MT. Consumer attitude and acceptance toward fish fed with insects: a focus on the new generations. *J Insects Food Feed*. 2022;8(11):1249-1263. doi:10.3920/jiff2021.0109
145. Bimbo F, Viscecchia R, De Devitiis B, Seccia A, Roma R, De Boni A. How do Italian consumers value sustainable certifications on fish? – An explorative analysis. *Sustainability*. 2022;14(6):3654. doi:10.3390/su14063654
146. Budhathoki M, Zolner A, Nielsen T, Rasmussen MA, Reinbach HC. Intention to buy organic fish among Danish consumers: Application of the segmentation approach and the theory of planned behaviour. *Aquaculture*. 2022;549:737798. doi:10.1016/j.aquaculture.2021.737798
147. Cerveira I, Baptista V, Teodósio MA, Morais P. What's for dinner? Assessing the value of an edible invasive species and outreach actions to promote its consumption. *Biological Invasions*. 2022;24(3):815-829. doi:10.1007/s10530-021-02685-3
148. Ferfolja M, Cerjak M, Matulić D, Maksan MT. Consumer knowledge and perception about fresh fish from organic farming in Croatia. *Ribar Croat J Fish*. 2022;80(1):7-16. doi:10.2478/cjf-2022-0002
149. Flores RMV, Widmar NO, Quagrainie K, Preckel PV, Pedroza Filho MX. Establishing linkages between consumer fish knowledge and demand for fillet attributes in Brazilian supermarkets. *J Int Food Agribus Mark*. 2022;34(4):368-388. doi:10.1080/08974438.2021.1900016

150. Güney OI, Özsahinoglu I, Erçen Z, Yeldan H, Dikel C, Sangün L. The mediator role of attitudes in fish choice behavior: A Turkish market survey. *Foods*. 2022;11(20):3180. doi:10.3390/foods11203180
151. Ho SS, Tan WQ, Chuah ASF, Chua PYY, Lau KXL, Chan JZA. Feed for thought: Factors predicting public support for funding and labeling preferences of alternative aquafeed. *Environ Commun*. 2022:1-19. doi:10.1080/17524032.2022.2051577
152. Hoerterer C, Petereit J, Krause G. Informed choice: The role of knowledge in the willingness to consume aquaculture products of different groups in Germany. *Aquaculture*. 2022;556:738319. doi:10.1016/j.aquaculture.2022.738319
153. Hoque MZ, Myrland Ø. Consumer preference for fish safety inspection in Bangladesh. *Aquaculture*. 2022;551:737911. doi:10.1016/j.aquaculture.2022.737911
154. Hoque MZ, Bashar MA, Akhter F. Ambiguity tolerance and confusion avoidance in the intent to purchase farmed fish. *J Appl Aquac*. 2022;34(3):564-597. doi:10.1080/10454438.2021.1872462
155. Hoque MZ, Akhter N, Chowdhury MSR. Consumers' preferences for the traceability information of seafood safety. *Foods*. 2022;11(12):1675. doi:10.3390/foods11121675
156. Hoque MZ, Sultana N, Haque A, Foisal MTM. Personal and socioeconomic factors affecting perceived knowledge of farmed fish. *J Agric Food Res*. 2022;8:100310. doi:10.1016/j.jafr.2022.100310
157. Hossain KZ, Xue J, Rabbany MG. Consumers' willingness to pay (WTP) for HACCP certified frozen farmed fish: A consumer survey from wet markets in Dhaka, Bangladesh. *Aquac Econ Manag*. 2022;27(1):143-158. doi:10.1080/13657305.2022.2046204
158. Kralik B, Weisstein F, Meyer J, Neves K, Anderson D, Kershaw J. From water to table: A multidisciplinary approach comparing fish from aquaponics with traditional production methods. *Aquaculture*. 2022;552:737953. doi:10.1016/j.aquaculture.2022.737953
159. Krešić G, Dujmić E, Lončarić D, Zrnčić S, Liović N, Pleadin J. Profiling of Croatian consumers based on their intention to consume farmed fish. *Foods*. 2022;11(14):2158. doi:10.3390/foods11142158
160. Masi M, Di Pasquale J, Vecchio Y, Pauselli G, Tribilustova E, Adinolfi F. A cross-sectional study in Mediterranean European countries to support stakeholders in addressing future market demands: Consumption of farmed fish products. *Aquac Rep*. 2022;24:101133. doi:10.1016/j.aqrep.2022.101133

161. Mehar M, Mekkawy W, McDougall C, Benzie JAH. Preferences for rohu fish (*L. rohita*) traits of women and men from farming households in Bangladesh and India. *Aquaculture*. 2022;547:737480. doi:10.1016/j.aquaculture.2021.737480
162. Peiró-Signes A, Miret-Pastor L, Galati A, Segarra-Oña M. Consumer Demand for Environmental, Social, and Ethical Information in Fishery and Aquaculture Product Labels. *Front Mar Sci*. 2022;9doi:10.3389/fmars.2022.948437
163. Petereit J, Hoerterer C, Krause G. Country-specific food culture and scientific knowledge transfer events – Do they influence the purchasing behaviour of seafood products? *Aquaculture*. 2022;560:738590. doi:10.1016/j.aquaculture.2022.738590
164. Pupavac SM, Jovanovic GK, Linsak Z, Glad M, Traven L, Zezelj SP. The influence on fish and seafood consumption, and the attitudes and reasons for its consumption in the Croatian population. *Front Sustain Food Syst*. 2022;6:945186. doi:10.3389/fsufs.2022.945186
165. Rickard LN, Houston CL, McGreavy B, Johnson BB, Gurney G. Fish prisons and bluehouses: Perceived risks and benefits of land-based aquaculture in four US communities. *Environ Commun*. 2022:1-17. doi:10.1080/17524032.2022.2062020
166. Saidi A, Sacchi G, Cavallo C, et al. Drivers of fish choice: an exploratory analysis in Mediterranean countries. *Agric Food Econ*. 2022;10(1):29. doi:10.1186/s40100-022-00237-4
167. Sigurdsson V, Larsen NM, Pálsdóttir RG, Folwarczny M, Menon RGV, Fagerstrøm A. Increasing the effectiveness of ecological food signaling: Comparing sustainability tags with eco-labels. *J Bus Res*. 2022;139:1099-1110. doi:10.1016/j.jbusres.2021.10.052
168. Stancu V, Brunsø K, Krystallis A, Guerrero L, Santa Cruz E, Peral I. European consumer segments with a high potential for accepting new innovative fish products based on their food-related lifestyle. *Food Qual Prefer*. 2022;99:104560. doi:10.1016/j.foodqual.2022.104560
169. Tran N, Shikuku KM, Hoffmann V, et al. Are consumers in developing countries willing to pay for aquaculture food safety certification? Evidence from a field experiment in Nigeria. *Aquaculture*. 2022;550:737829. doi:10.1016/j.aquaculture.2021.737829
170. Wongprawmas R, Sogari G, Gai F, Parisi G, Menozzi D, Mora C. How information influences consumers' perception and purchasing intention for farmed and wild fish. *Aquaculture*. 2022;547:737504. doi:10.1016/j.aquaculture.2021.737504

171. Abaidoo E, Malone T, Melstrom RT. Fish demand in the US Great Lakes region in the face of seafood mislabeling. *Aquac Econ Manag*. 2023;27(4):666-692. doi:10.1080/13657305.2023.2167019
172. Baldi L, Trentinaglia MT, Peri M, Panzone L. Nudging the acceptance of insects-fed farmed fish among mature consumers. *Aquac Econ Manag*. 2023:2265875. doi:10.1080/13657305.2023.2265875
173. Baraibar-Diez E, Llorente I, Odriozola MD. Text emotion analysis in aquaculture communication via Twitter: The case of Spain. *Mar Policy*. 2023;152:105605. doi:10.1016/j.marpol.2023.105605
174. Beyer L, Chan C, LaPorte P, Lee CS. Assessing high school students' perceptions and preferences for aquaculture versus wild-caught seafood. *J World Aquac Soc*. 2023;54(4):801-813. doi:10.1111/jwas.12949
175. Bogliacino F, Charris R, Codagnone C, et al. Less is more: Information overload in the labelling of fish and aquaculture products. *Food Policy*. 2023;116:102435. doi:10.1016/j.foodpol.2023.102435
176. Cantillo J, Martín JC, Román C. Understanding consumers perceptions of aquaculture and its products in Gran Canaria island: Does the influence of positive or negative wording matter? *Aquaculture*. 2023;562:738754. doi:10.1016/j.aquaculture.2022.738754
177. Fong CR, Gonzales CM, Rennick M, et al. Conflict and alignment on aquaculture among Californian communities. *Aquaculture*. 2023;580doi:10.1016/j.aquaculture.2023.740230
178. Forleo MB, Palmieri N. Environmental attributes of wild versus farmed tuna: Beliefs, knowledge and purchasing choices of Italian consumers of canned tuna. *Sustainability*. 2023;15(9):7149. doi:10.3390/su15097149
179. Forleo MB, Romagnoli L, Palmieri N. Are Italian consumers of canned tuna fish sensitive to environmentally sustainable product attributes? *Br Food J*. 2023;125(2):608-625. doi:10.1108/bfj-08-2021-0893
180. Hossain A. Quantity or quality of fish in a developing country: A hedonic analysis. *J Appl Aquac*. 2023;35(2):394-409. doi:10.1080/10454438.2021.1970687
181. López-Mas L, Claret A, Arvisenet G, et al. European consumers' beliefs about the main pillars of the sustainability: a comparison between wild and farmed fish. *Aquac Int*. 2023;31(4):2035-2055. doi:10.1007/s10499-023-01070-2
182. Menozzi D, Wongprawmas R, Sogari G, Gai F, Parisi G, Mora C. The role of objective and subjective knowledge on the attitude and intention of Italian consumers to purchase farmed and wild fish. *Agric Food Econ*. 2023;11(1)doi:10.1186/s40100-023-00288-1

183. Nguyen L, Gao ZF, Anderson JL. Perception shifts in seafood consumption in the United States. *Mar Policy*. 2023;148:105438. doi:10.1016/j.marpol.2022.105438
184. Olsen MS, Amundsen VS, Osmundsen TC. Exploring public perceptions and expectations of the salmon aquaculture industry in Norway: A social license to operate? *Aquaculture*. 2023;574:739632. doi:10.1016/j.aquaculture.2023.739632
185. Rosenau S, Wolgast T, Altmann B, Risius A. Consumer preference for altered color of rainbow trout (*Oncorhynchus mykiss*) fillet induced by Spirulina (*Arthrospira platensis*). *Aquaculture*. 2023;572:739522. doi:10.1016/j.aquaculture.2023.739522
186. Zheng Q, Nayga RM, Yang W, Tokunaga K. Do U.S. consumers value genetically modified farmed salmon? *Food Qual Prefer*. 2023;107:104841. doi:10.1016/j.foodqual.2023.1048

6. DISCUSSION AND FINAL CONSIDERATIONS

6.1. Blood hydrolysates as aquafeed ingredients

In this thesis, we evaluated the incorporation of functional ingredients to address the adverse effects commonly associated with diets high in plant ingredients. These effects may include compromised feed intake, digestibility, growth, stress response, and immunity status. Following a sustainability and circular economy approach, we considered agrifood industry by-products as sources of such functional ingredients: swine blood from slaughterhouses and sardine cooking wastewaters from a canning industry. Both are low value residues, which could be valorised through processing using novel technologies.

Raw swine blood predominantly contains protein as its primary macronutrient with levels typically ranging between 91% and 95%, in dry basis (Márquez et al., 2005; Sorapukdee & Narunatsopanon, 2017). This makes it an interesting candidate for processing through hydrolysis. This process has several advantages: it breaks down complex proteins, amino acids (AA) become more easily available, and often protein digestibility is increased (Sandbakken et al., 2023; Zhang et al., 2022). Furthermore, bioactive peptides, that are generally encoded amidst the protein sequences, can be obtained and used as functional ingredients in aquafeeds. In the market, some hydrolysates from swine by-products, including blood or its components, are already available (table 1). In general, these substances find greater application in enhancing palatability in piglet diets, with relatively limited exploration in the realm of aquafeeds. Additionally, their bioactivity remains largely unexplored or minimal.

Table 1 – Swine hydrolysates available in the market.

Commercial name	Ingredient description	Models tested	Ref.
HSLP	Enzymatically hydrolysed swine liver protein produced and supplied by Brasil Foods S/A (sector BRF® Ingredients)	Nile tilapia	dos Santos Cardoso et al. (2021)
HSMP	Enzymatically hydrolysed swine mucus protein produced and supplied by Brasil Foods S/A (sector BRF® Ingredients)	Nile tilapia	dos Santos Cardoso et al. (2021)
Pepton 85/16	Enzymatically hydrolysed pig haemoglobin obtained through hydrolysis of red blood cells under alkaline conditions, produced by APC Europe, S.A., Catalonia, Spain	Strawberry plant (cultivar Diamante ®), lettuce (cultivar Arena)	Marfà et al. (2009); Polo et al. (2006)

HPM	Hydrolysed porcine mucosa produced by Yichang Huatai Biological Technology Co., Ltd., Yichang, China	hybrid grouper	Yang et al. (2021)
PHC	Porcine hydrolysed collagen produced by Sonac, Son, The Netherlands.	Thoroughbred horse	Camacho-Luna et al. (2022)
Palapro	A combination of swine plasma and intestinal peptones produced by Sonac, Son, The Netherlands.	Piglets	Sonac (2024c)
MucoPro 80P	Hydrolyzed porcine peptone product, produced from intestinal mucosal tissue that is collected from dedicated pig slaughterhouses. This mucosal tissue is enzymatically hydrolysed to extract heparin. The hydrolysed mucosa undergoes further processing steps like separation, desalination, concentration and spray-drying.	Piglets, calfs	(Sonac, 2024b)
Innomax MPI	Spray-dried swine protein produced via a thermal hydrolyzation process.	Cats, dogs	(Sonac, 2024a)
Porcine whole-blood protein hydrolysate	Hydrolysate produced by AminoLab Co., Ltd., (Seoul, Korea). Coagulated blood was liquified and treated with food grade serine protease. Then, incubation with activated carbon powder. Then, hydrolysate passed 3x through filters of diatomaceous earth. Filtrate was sterilized thermally and spray-dried.	ICR mouse	Jin et al. (2022)
Palbio 50 RD	Hydrolysed porcine mucosa produced by Bioibérica, Spain. Obtained from porcine digestive mucosa after heparin separation, through enzymatic hydrolysis, followed by solubilization and sterilization. Then, the hydrolysate is sprayed into a soybean meal carrier and dried with a fluid bed system.	Broilers, piglets	Mateos et al. (2014); Middelkoop et al. (2023)
Palbio 62 SP	Spray-dried enzymatic hydrolysate from porcine intestinal mucosa produced by Bioibérica, Spain.	Piglets	Corassa et al. (2007); Solà-Oriol et al. (2011)
Bone protein hydrolysate	Collected directed from [unknown] producers. Bone pulp was treated with lactic acid and alkaline solutions at 50 - 150 °C. Then, hydrolysis at 130 -140 °C and 0.3-0.4 MPa, followed by decanting at 90 °C for fat separation. The final product was dried.	Broilers	Jamroz et al. (2011)
Cravings 850	Enzymatically hydrolysed protein derived from plasma, developed for enhanced palatability and digestibility, produced by APC	Unknown	APC (2024)
Pepteiva	Hydrolysate obtained from pig plasma produced by APC Europe	Gilthead seabream, meagre	Fernández-Alacid et al. (2021); Gisbert et al. (2021)
NORLAN LX	Pig blood protein hydrolysate produced by Proalan SA, Spain	Gilthead seabream	Gisbert et al. (2012)
Liver extract liquid	Produced by A. Costantino Bioactive Peptides, from swine liver	Unknown	A. Costantino (2023)
Heme Iron	Dark powder obtained from selective hydrolysis of haemoglobin. Produced by A. Costantino Bioactive Peptides	Unknown	A. Costantino (2023)
Placenta extract	Obtained by specific hydrolysis under controlled parameters, extracting peptides in aqueous solution and mild conditions. Produced by A. Costantino Bioactive Peptides	Unknown	A. Costantino (2023)

In Chapter 3.1, swine blood hydrolysates were developed under the scope of the MobFood project (MobFood Consortium, 2017), being obtained using a *Cynara cardunculus* extract, followed by fractionation using a membrane system. Although these high-quality hydrolysates targeted human consumption, we have hypothesized that, in that process, the retentates of the filtrations applied could still possess bioactive peptides with valuable properties that could be used for plant-based diets for European sea bass. In this sense, we tested the retentate of both micro-filtration (RMF-H) and nano-filtration (RNF-H). In parallel, another hydrolysate, the AH-H, was obtained through a process called auto-hydrolysis and no further fractionation was applied at the end. Auto-hydrolysis requires subcritical water, instead of requiring enzymes to break down the proteins and release the bioactive peptides. In this way, the cost to obtain this hydrolysate can be minimized.

The study evaluated the impact of these hydrolysates on various aspects of fish, including growth performance, digestibility, intestinal morphology, muscle development, infectious response and stress response. This comprehensive approach aimed to cover a wide range of possible functionalities of these hydrolysates. The three hydrolysates evidenced distinct functional properties.

Overall, the most promising hydrolysate to be added to plant-based diets for European seabass was the RNF-H. Fish fed the diet containing this hydrolysate for 74 days exhibited a final weight and body length that were similar to those fed on a non-supplemented plant-based (NC) control, although still slightly below the levels observed in the positive control (PC, fishmeal-based diet). This can be due to this diet's impact on lipid and energy digestibility, compared to the PC. Particularly, this diet led to a lower digestible energy, mostly as a consequence of a worse lipid digestibility and higher faecal losses of both protein and lipids. Additionally, another work by our group with these hydrolysates showed that RNF-fed fish displayed a down-regulation of fibroblast growth factors (FGFs) *fgf4* and *fgf6* and calpain 1 in the muscle, compared to the PC, which may also partially contribute to the growth performance differences between these two diets. (Velasco et al., 2023). Contrary to RNF, the RMF diet displayed the worst overall growth performance of all diets. This diet hindered lipid and dry matter digestibility. Moreover, mineral digestibility, including calcium and potassium, was also diminished. Accordingly, the intestinal morphology of fish fed this diet was affected, with fish displaying a lower *villus* length and *muscularis externa* compared to the control diets. Indeed, the compromised intestinal morphology may be responsible for the poorer digestibility, resulting subsequently in a worse growth performance. Furthermore, fish fed the RMF diet showed a tendency to have lower plasmatic protein levels, as shown in chapter 3.2. although this was not significant. Considering that protein deposition is directly correlated with the fish growth (Dumas et al., 2007), having lower available protein for deposition may have hindered growth. This hydrolysate also affected muscle cellularity, leading to a lower number of small sized muscle fibres (diameter below 40 μm), which could be a consequence of a down-regulation of *myod1,* the myoblast determination factor (Velasco et al., 2023). This could have affected the fish growth as well. Moreover, the fibroblast growth factor *fgf4* was also down-regulated, contributing as well to the poorer growth. In any case, the fish displayed a compensatory muscular growth mechanism, through the down-regulation of *capn1* and an up-regulation of *mymk*, which suggest a decrease in muscle proteolysis but increase in myoblast fusion (Velasco et al., 2023). However, the compensatory growth mechanisms were not enough, and this diet led to the worst growth performance. Finally, the AH-H did not stand out in any of the analysed parameters, displaying generally an intermediate performance between RMF-H and RNF-H. Moreover, the growth performance was worse when compared to both a FM-based diet and a plant-based, non-supplemented, diet. This contrasts with the expression of growth factors and genes related to myogenesis in the muscle of fish fed AH, which are statistically similar to those fed both controls (Velasco et al., 2023). Other swine hydrolysates available in the market have also been shown to affect farmed fish growth performance. While in meagre (*Argyrosomus regius*), diets with Pepteiva, an hydrolysate obtained from porcine plasma produced by APC Europe, impaired growth performance (Fernández-Alacid et al., 2021), the same results were not verified in gilthead seabream (*Sparus aurata*), for which growth was improved (Fernández-Alacid et al., 2022; Gisbert et al., 2021). Another pig blood protein hydrolysate, NORLAN LX, produced by Proalan SA, was able to replace CPSP90 (a fish protein hydrolysate) in diets for gilthead seabream larvae without altering larval growth and development (Gisbert et al., 2012). These results suggest a wide variation in fish response depending on the hydrolysate and the species where it was tested.

The hydrolysates available in the market are sometimes reported as having benefits regarding their antioxidant or stress mitigating properties (Table 1). For example, Peptone 85/16 is an enzymatic hydrolysate obtained from pig haemoglobin, produced by APC Europe which has shown promising results in the mitigation of plant thermal stress (Marfà et al., 2009; Polo et al., 2006). In this sense, a stress challenge (air exposure followed by recovery in different tanks) was also performed in this thesis (chapter 3.2) to assess if the selected swine hydrolysates could help fish cope with the stressful conditions of fish farms. However, in general, diets with the hydrolysates did not show significant improvement in the plasmatic markers, after this stress challenge. In any case, all hydrolysates showed the potential to mobilize non-enzymatic antioxidants after the stress event, as evidenced by the higher levels of the total antioxidant capacity

(TAC) in the liver. This was not enough to cause a significant impact on the lipid peroxidation levels of this tissue, suggesting that to promote this effect, a different concentration of the hydrolysates should have been applied. Overall, once more, RNF seems to perform better than the remaining supplemented diets. Indeed, the RMF diet was associated with higher superoxide dismutase (SOD) and total glutathione (TG) levels in the liver. This could suggest a higher oxidative pressure caused by this diet, leading to the need to increase enzymatic antioxidant activity to counteract it. Similarly, the AH diet required increased activities of glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST). This observation implies that the diet may not effectively regulate antioxidant defences or may promote oxidative stress itself, leading to heightened requirements for these enzymes to uphold homeostasis. This phenomenon occurred despite the presence of low-sized (<13.7 kDa) peptides in the hydrolysate. In contrast, the RNF diet, which also possesses low-sized peptides, did not show these negative effects. A limited bioavailability of such peptides could have accounted for the diet's lack of efficacy to prevent oxidative stress. Notably, on chapter 3.1., we demonstrated that the AH compromised protein digestibility and elevated faecal nitrogen losses, impacting not only protein metabolism related to growth but also diminishing the availability of bioactive peptides. Alternatively, this could be due to the presence of specific peptides in the AH-H which are not present in RNF-H, as a consequence of the different hydrolysis process applied or the absence of post-hydrolysis fractionation.

The best result obtained with the RNF-H refers to its potential to prevent mortality after infection with *T. maritimum*. The RNF diet showed significantly less mortality than the NC diet, and it was also lower than that observed for the PC, even though that difference was not significant. This result is very significant, considering that *T. maritimum* is a bacterial species that provokes high mortality and substantial losses for fish farms. This has led us to submit an international patent application regarding the development and application of the RNF hydrolysate (Pintado et al., 2022). In contrast, the RMF diet was associated with a decrease in plasma lysozyme activity, a significant marker of the innate immune system, and, subsequently, with a higher mortality rate when faced with an infectious challenge with *T. maritimum.* Comparing these results with those obtained for the RNF, it is clear that the microfiltration is essential to either remove possible unwanted contaminant microorganisms, but also bigger protein aggregates that are not soluble, despite the cost associated with these methods. Interestingly, the mortality of fish fed the AH diet when infected with *T. maritimum* was slightly lower than the non-supplemented control (although still statistically similar) and equal to a FM-based one. It is possible that the peptides responsible for the activity against *T. maritimum* are still present in the AH-H, but in a lower concentration, due to the lack of fractionation. Therefore, in the future, it is suggested that the membrane process applied for obtaining RNF-H is applied to AH-H, and the inclusion of the obtained fractions in aquafeeds tested for potential bioactivity. In theory, this would concentrate bioactive peptides responsible for immunity improvement and muscle development observed in this diet, and possible removal minerals or other compounds that hinder oxidative homeostasis.

Some of the swine hydrolysates available in the market may also promote fish immunity (Table 1). Pepteiva improved mucus antibacterial activity in both meagre and gilthead seabream (Fernández-Alacid et al., 2021; Gisbert et al., 2021), suggesting a possible modulation of the immune system. As such, the results obtained for meagre using Pepteiva appear to be in line with those obtained in our work for the RNF-H. However, the aforementioned studies using Pepteiva were not validated through infectious challenges, and mucus antibacterial activity was not tested against *T. maritimum,* and thus their efficacy remains to be proved.

It is worth considering that vaccination for European seabass against this species is not available; the only commercially available vaccine is approved for turbot and its efficacy on other fish species has been deemed as low (Mabrok et al., 2023). Thus, beyond treatment with antibiotics, the only alternative is the usage of autogenous bacterins, which are inactivated or killed bacteria from a particular outbreak, injected to promote immunization during an outbreak (Khalil et al., 2018; Mabrok et al., 2023). The development of alternatives that could prevent the mortality of fish facing tenacibaculosis would then be of the highest interest, considering not only animal welfare, but also the 'one health' concept, minimizing the need for antibiotics and diminishing the spread of antimicrobial resistance. In addition, this would ease one of the most common concerns by consumers regarding farmed fish, as observed in chapter 5: the usage of chemicals such as antibiotics. Diets containing the RNF-H could therefore be used preventively, when an outbreak is feared: for example, near periods of transportation of the animals, or with higher temperatures and salinities, which have been associated with higher prevalence and severity of *T. maritimum* outbreaks (Avendaño-Herrera et al., 2006; Mabrok et al., 2023). In this way, possible impacts to the animals' growth and digestibility would be minimal, and in addition, the costs associated with a diet supplemented with a functional ingredient would be minimized. Indeed, one of the issues regarding the application of hydrolysates in aquafeeds refers to its price. Obviously, economic viability of this depends on the capacity to produce the hydrolysate at a significant scale, along with the benefits that it brings to the farms. However, the hydrolysate market for animal feed applications is growing. The increasingly stringent regulations regarding antibiotic therapies drive the market development as hydrolysates' functional properties become more established, aided by the fact that they can be obtained from by-products. Nonetheless, in this matter, the developed hydrolysates could not be used considering a halal food chain supply, as they are a swine by-product.

A SWOT (strengths, weaknesses, opportunities and threats) analysis was performed considering the RNF-H hydrolysate (Figure 2). The strengths of this product include the ability to increase European seabass resistance to a pathogen when included in a plant-protein based diets and the upcycling of a low-valued product from the agri-food industry (swine blood) into a high-valued one with functional properties. Furthermore, the RNF-H itself is a co-product of an ingredient targeted for human nutrition with high market potential: after the nanofiltration, the RNF-H corresponds to the retentate, but the filtrate is also rich in bioactive peptides and has been proposed as valuable ingredient for human nutrition (chapter 3.1, figure 1), due to its anti-hypertensive and antioxidant properties (Araújo-Rodrigues et al., 2022). In contrast, the identified weaknesses include the lipid digestibility impairment, potentially affecting fish performance over the long term, along with the costs associated with the required membrane processing and further drying. However, these drawbacks could be mitigated by employing the diets sparingly in scenarios anticipating infectious outbreaks, as aforementioned. Also, leveraging renewable energy sources could play a significant role in reducing drying costs. In any case, opportunities emerge: the growing market for functional feeds as a result of increasing consumer and legislative pressure to minimize the use of antibiotics and improve animal welfare, along with lower availability of fishmeal that adds pressure to supplement plant diets with bioactive compounds to ensure fish welfare and physiological status – all of these can be addressed with the application of the RNF-H. Finally, regarding possible threats, consumer acceptance of the usage of blood derivatives in feed can be an issue. In addition, the religious ban regarding swine products in the Middle East (Halal feeds) minimizes the application of the hydrolysates in many countries. Legislation instability in more conservative regions regarding the usage of animal by-products for animal feeds may also hinder the applicability of this hydrolysate.

Overall, despite the existence of several swine hydrolysates in the market, they are often used in piglet diets and underexplored in aquafeeds. Moreover, most of these are suggested as palatability enhancers by the producing companies and their bioactivity is either underexplored or minimal. Therefore, the developed RNF-H has potential to further exploration, considering its benefits regarding enhanced fish immunity.

Figure 1 – SWOT Analysis regarding RNF-H.

6.2. Aromas from the canning industry as aquafeed ingredients

Literature reports show that, generally, replacing FM with plant proteins tends to decrease diet palatability and VFI for several carnivorous species (Dias et al., 1997; Liang et al., 2019; Sabioni et al., 2022; Torstensen et al., 2008). This reduction in feed intake can occur at a short-term period, followed by habituation to the diet, where fish eat to fulfil their nutritional needs despite the low palatability of diets (Liang et al., 2019). However, even a short-term decrease in feed intake can impact the overall growth performance of animals (Torstensen et al., 2008). In addition, uneaten feed poses a problem for fish farms due to economic losses and environmental impacts related to poorer water quality (Amirkolaie, 2011; Hu et al., 2021). Moreover, it is possible that increasing diet palatability, fish will eat beyond the bare necessities of nutrients and energy, and this would in turn lead to an improved growth performance.

Thus, the aquaculture sector resources to the supplementation of plant-based diets with feed attractants in order to minimize the reduction in diet palatability. In a manner similar to our approach in developing the hydrolysates, we adopted a circular economy perspective, postulating that these attractants could be derived from by-products of the agrifood industry. As such, in this work, we hypothesized that volatile compounds extracted from the canning industry wastewaters could perform that role in plant-based aquafeeds for European seabass. While several processes have been developed for the treatment of these wastewaters (Cao et al., 2022; Venugopal & Sasidharan, 2021), generally, these do not allow for the recovery of the odorous volatiles that confer them their characteristic “fish smell”. Volatiles from oyster processing wastewaters and crab boiling juice have been obtained from pervaporation (Martínez et al., 2014; Soares et al., 2020), while nanofiltration and osmotic evaporation have been applied to recover aromas from shrimp cooking juice (Jarrault et al., 2017). Reverse osmosis coupled with electrodialysis was applied to concentrate an industrial mussel cooking juice (Cros et al., 2004). However, these extracts have compounds generally classified as off-flavours, such as sulphur-based compounds, or almond flavours such as benzaldehyde. Moreover, to the best of our knowledge, the valorisation of these volatiles as feed attractants has never been studied.

In chapter 4.1., a method for the recovery of aromas from sardine cooking wastewaters was developed. The cooking wastewaters were collected and supplemented with an acorn antioxidant extract. This was done to prevent degradation of the volatile compounds. Subsequently, a reverse osmosis step was employed to further purify the solution. Then processing through vacuum distillation or liquid-liquid extraction with soybean oil took place. Soybean oil was the chosen oil due to its widespread use in aquafeeds, potentially offering additional benefits in enhancing palatability and acceptance by aquatic species. Using these two different methods allowed the comparison between two aroma-rich fractions: one oil-based, another one water-based. These fractions had a significantly different volatiles' profile, depending on the hydrophobicity of the compounds. Furthermore, we expected to achieve two main objectives with the overall processing: to remove off-flavours and to concentrate compounds reported to have a fish odour, such as 1-penten-3-ol. The removal of off-flavours was successful – sulphur compounds and alkanes were only detected in the original cooking waters (CW-A). However, we observed losses in 1-penten-3-ol and aldehydes, when comparing CW-A to the aroma extracts produced from vacuum distillation (VD-A) and liquid-liquid extraction (LLE-A). As such, further improvement in both processes should be performed if we aim at concentrating these compounds. Comparing the two processes, vacuum distillation led to a higher concentration of 1-penten-3-ol and 2,4-heptadienal and thus appears more promising. In contrast, the LLE-A displayed higher concentrations of organic acids.

The impact of these extracts on the modulation of feed intake was evaluated both at a short and long term. The reason for this lies on the different mechanisms that regulate intake at each term, and the different factors that affect it. In a short-term trial, diet palatability plays a very strong role in feed acceptance or rejection. Therefore, the regulation of feed intake is mostly mediated by hedonic (pleasure-driven mechanisms). In contrast, at a longer term, nutrient or energy needs become more significant, along the effect of habituation to the diets. Consequently, in this case, homeostatic regulation of intake is more relevant, although hedonic regulation keeps affecting intake. In addition, we tested not only the developed extracts after processing, but also the inclusion of the unprocessed sardine cooking waters (supplemented only with the antioxidant), in order to assess whether the processing was beneficial in terms of the intake modulation properties of the extracts.

In chapter 4.2., after an 18-week trial, the diet with the highest VFI was the LLE diet, although none of the diets were able to significantly increase VFI compared to the control, a non-supplemented plant-based diet. CW displayed the lowest VFI, being significantly lower than the LLE diet. A possible explanation for this is the presence of off-flavours in the CW-A, including alkanes and sulphur compounds, and "rubber"-smelling compounds, including pentanal and 2,4-heptadienal (Venkateshwarlu et al., 2004). Moreover, the LLE-A showed the highest amounts of short-chain organic acids of all extracts, which have been reported to stimulate intake in common carp (Kasumyan &

Døving, 2003), along with hexanal, which is also associated with a fish odour (Giri et al., 2010). However, in this trial, we observed that metabolism was affected by dietary treatments and thus homeostatic regulation was probably quite significant in the regulation of feed intake. In this sense, we observed that LLE led to the highest feed conversion ratio (FCR) of all diets and lowest protein efficiency ratio (PER), along with a reduction in circulating nutrients 6 h after feeding, suggesting a lower available energy for fish fed LLE. This could have triggered the increase of intake to maintain nutrient homeostasis, resulting in similar final weight between all dietary treatments.

Considering the uncertainty regarding the impact of the extracts on the modulation of the hedonic regulation of feed intake, a short-term trial was then performed (chapter 4.1.). Feed intake quantification and fish sampling took place after a single first meal with the experimental diets (plant-based non-supplemented control and the diets supplemented with the aroma extracts). Contrarily to what occurred at the long trial, decreased intake was observed in all supplemented diets compared to the non-supplemented control. Bearing in mind that all fish had a similar metabolic state, having all been fed the same diet during quarantine and habituation to the system, the most likely scenario is that the hedonic regulation was more prominent, which suggests a lower palatability of the supplemented diets. The results obtained for the plasma metabolites corroborate this hypothesis, as in the short-term trial, no significant differences in any of the analysed parameters were found. In contrast, at the end of the long-term trial, significant differences were observed in plasma glucose and liver glucose and triglycerides. Overall, it seems that at the short-term, the fish rejected the supplemented diets, due to a hedonic regulation of feed intake. In a longer trial, fish displayed habituation to the diets and the nutrient or energy needs significantly affected the feed intake. Curiously, the expression of intake-regulating neuropeptides was not affected by dietary treatments in neither trial. The regulation of feed intake relies on the intricate integration of various neuropeptides and hormones. Consequently, even non-significant changes in the individual expression of neuropeptides can lead to significant alterations in the overall control of feed intake. In the short-term trial, the reduction of intake may have been due to a combination between a decrease in the expression of the orexigenic agouti-related peptide (*agrp*) in the telencephalon, and the increase in anorexigenic cocaine and amphetamine regulated transcript (*cart*) and pro-opiomelanocortin (*pomc*) in both the telencephalon and hypothalamus. In contrast, VFI was positively correlated with the expression of neuropeptide Y (*npy*) in the hypothalamus, in the longer trial. The cascade of signals regulating intake caused by the homeostatic regulation may have resulted in a higher expression of this orexigenic peptide, leading to a higher VFI. Moreover, in this trial, the neuropeptide expression pattern in the telencephalon was different compared to that of the hypothalamus, with less variation observed in response to diets. This further supports the idea that in this trial, homeostatic, rather than hedonic, regulation was prevailing: the hypothalamus has been more associated with the first, while the reward-based pathways are most likely located in the telencephalon (Comesaña et al., 2018).

Although the negative impact of the extracts on the hedonic regulation of VFI was not expected, several factors may help explain this. Firstly, fish's response to odorants may differ from that of mammals, and thus not elicit the expected response to the fish odour. Additionally, the majority of the volatile compounds found in the examined extracts stem from the degradation of fish oil during sardine cooking (Ferraro et al., 2013). Consequently, while these compounds may carry a "fish" odour to humans, they could be perceived by fish as linked to rancidity rather than the scent of fresh fish, potentially serving as a deterrent. In a similar fashion, while extracts removed what is generally considered an “off-flavour” to humans, further studies regarding the response of the animals to individual compounds may shed light into what the animals consider an attractant and a deterrent, aiding in a better optimization of the extraction processes.

In addition to the evaluation of the impact of the aromas on feed intake, organoleptic characteristics of the fish were also assessed in chapter 4.2. This was done because of the possibility of flesh flavour modulation due to the aromas. Furthermore, when the fish metabolism is affected, as occurred with the LLE diet, there is a risk of altering the texture and flavour of the fillet (Izquierdo et al., 2005; Izquierdo et al., 2003; Matos et al., 2012). Finally, plant-based diets have been reported to have negative effects on texture, skin colour and flavour of European seabass (Albendea et al., 2023; Pleić et al., 2022). Thus, we conducted a thorough analysis, involving both an instrumental characterization of colour and texture and a sensory analysis with a consumer panel. For this, we employed the Rate-all-that-apply (RATA) methodology, which involves presenting a questionnaire to a consumer panel containing a list of terms for evaluation. They are then asked to select all the terms they believe are applicable to characterize the sample and for the selected terms, to rate their intensity with a structured scale. This is an improvement to the CATA (Check-all-that-apply), where the questionnaire only presented the items for selection without rating. Consequently, the RATA methodology is more discriminative (Ares et al., 2014).

Overall, we only observed significant differences in the texture of fillets of fish fed LLE, with a significant increase in instrumentally assessed hardness. However, this was not perceived by the sensory panel, with all samples being well accepted – all diets had an average overall liking equal or above to 7.5 in a 1 to 9 scale. This is particularly important because the dietary treatments had high levels of plant-based ingredients, which did not seem to compromise overall liking. Moreover, the use of different aromas, regardless of their impact on fish growth or nutrient utilisation, did not affect the sensory profile of European seabass by the consumer panel, even when a highly discriminative methodology such as RATA is employed.

In the literature, it is possible to see that most of the reports focusing of feed attractants use protein-based supplements, such as AA mixtures, marine meals or hydrolysates (see Table 4 of section 1.4). At a short term period (one week or less), jack mackerel meal and FM hydrolysate seem to increase feed intake for olive flounder (*Paralichthys olivaceus*) and Senegalese sole (*Solea senegalensis*), suggesting a modulation of the hedonic regulation of intake (Barroso et al., 2013; Jeong et al., 2020). Other studies also showed an increased intake at longer term by supplementing plant-based diets with AA mixtures, marine meals or hydrolysates. For example, Dias et al. (1997) observed that an AA mixture increased feed intake, feed efficiency and weight gain for European seabass fed a plant-based diet, to levels similar to of a FM-based diet, over 21 days. Baek et al. (2021) also showed that a jack mackerel meal increased feed intake and weight gain of rockfish (*Sebastes schlegeli*), while maintaining FCR, in an 8-week trial. Nevertheless, not all hydrolysates and marine meals work for all species. Novriadi et al. (2017) showed that a squid meal and a squid hydrolysate did not affect feed intake of pompano (*Trachinotus carolinus*). Similarly, a blue mussel meal did not affect intake and weight gain of turbot (*Psetta maxima*), in a plant-based diet, and led to a worse performance compared to a FM-based diet. Moreover, Calo et al. (2024) showed that a umami feed attractant produced by Lucta, S.A, composed of peptides, AAs and nucleotides, did not affect feed intake of rainbow trout (*Oncorhynchus mykiss*), despite affect nutrient digestibility. These results suggest that intake regulating mechanisms and preferred attractants might vary greatly for specific compounds and for different species, in line with the research presented on chapters 4.1 and 4.2, emphasizing the need of further research. Overall, most studies still rely on protein-based feed attractants, with variable efficacy, while other compounds, such as alcohols, ketones, aldehydes and other organic compounds, remain largely unexplored.

A SWOT analysis was conducted for the LLE-A extract, as this displayed the best effect on voluntary feed intake (Figure 3). Regarding LLE-A strengths, its extraction can be performed in parallel with processes to clean the sardine cooking wastewater, allowing its reuse within the canning factory or its release without harmful consequences, thus having a beneficial environmental impact. Moreover, the extraction is performed with an oil (soybean) that is already used by the aquafeed industry, being widely available and allowing its simple incorporation into aquafeeds. Finally, fish fed the supplemented diets displayed high flesh quality for consumption. However, its weaknesses were that the benefit to the intake is limited and only visible at a long term, thus requiring further optimization. Additionally, FCR and nutrient utilisation in European seabass were impaired, which also needs to be addressed. In terms of the existing opportunities, these are mostly related to these processes being within a circular economy framework: addressing growing consumer and legislative pressure to maximize resource circularity and minimize waste. Additionally, the lower availability of fishmeal presents an opportunity to supplement plant-based diets with bioactive compounds ensuring the same efficacy while reducing reliance on marine resources. There is also a growing emphasis on reducing the discharge of waste into water streams, which is a strong concern of the canning industry. Finally, regarding threats, other attractants in the market may be more effective, such as those derived from marine ingredients or protein-based ones (hydrolysates and AA mixes) although this still remains an issue in plant-based aquafeeds for carnivorous fish.

Figure 2 – SWOT Analysis regarding LLE-A.

6.3. Consumer perception of aquaculture products

Overall, we aimed at understanding consumers' preferences regarding aquaculture products and promoting aquaculture growth, accordingly, considering the minimization of this sector's economic and environmental impacts.

An extensive literature review was conducted regarding consumer preferences regarding wild and farmed fish, on chapter 5. The analysed reports employed a multitude of methodologies to retrieve that information: personal interviews, focus groups, think aloud protocols, surveys, choice experiments, among others. No methodology is perfect. Personal interviews and think aloud protocols allow the interviewed person to deeply explain the reasons behind a particular choice or behaviour, to clarify any doubts regarding the questions being asked and to present their specific needs or apprehensions, allowing researchers to better understand the consumer's point of view (Arsel, 2017; Thelwall & Nevill, 2021; Williamson, 2013). A similar situation occurs with focus groups, which have the added benefit of stimulating ideas through group interaction (Stewart et al., 2007; Williamson, 2013). However, these types of data collection have some disadvantages, namely time constraints, variations caused by the interviewer behaviour and difficulties in collecting data from a representative sample (Onwuegbuzie et al., 2009). Questionnaires, particularly online ones, can overcome some of these issues. They allow the collection of high amounts of data with minimal cost (Williamson, 2013). Furthermore, respondents can choose the most convenient time for them to answer the survey and interviewer bias is minimized, because questions are posed in the same way, without visual cues affecting the response. However, respondents cannot clarify questions and complex questions, in general, cannot be posed through this method (Williamson, 2013). Choice experiments have been developed, in which a consumer is presented with two or more options of a product and is asked to choose one (Cantillo et al., 2020). They can be more or less realistic: the products can be real, and the experiment can take place in a shopping situation (Lizin et al., 2022; Olesen et al., 2010; Risius et al., 2017). However, most commonly, hypothetical products are presented, which, in any case, allow to assess the perception of attributes or products that are not yet in the market (Ankamah-Yeboah et al., 2018; Cantillo et al., 2020; Grimsrud et al., 2013; Lizin et al., 2022). In addition, selection of a panel that is representative of the population in study is essential, and it is evident that not all reports present that: for example, online surveys might lack the perspective of older respondents, which may not have easy access to the internet; other studies select only respondents from a certain group (from a university; from participants in knowledge transference events) (Hansen & Onozaka, 2011; Hoerterer et al., 2022; Petereit et al., 2022). This can compromise the viability of the obtained results. Moreover, these studies need to consider the social and temporal framework of respondents. As such, major events may lead to changes in consumer perception. For example, with the COVID-19 pandemic, consumers became more aware of food safety, while the issue of increasing prices and loss of purchase power due to inflation may lead to consumers to value the price attribute above others, minimizing their willingness to pay for specific attributes. Thus, consumer perception is not static, and should be frequently assessed.

Nevertheless, the data is overwhelmingly pointing in the same direction, from studies with diverse methodologies and using a multitude of consumer samples. Wild fish is still generally perceived as superior: consumers believe it has a better quality (freshness, texture, taste), less chemicals derived from feeding (such as antibiotics), a higher naturalness, is environmentally friendlier and has a better welfare.

While consumer misinformation, as evidenced by the low levels of both objective and subjective knowledge, significantly influences the perception of farmed and wild fish, some aspects stood out as significant for aquaculture improvement from the consumer's perspective.

In order to better captivate consumers, farmed fish should have excellent quality, namely in terms of appearance, taste, texture and odour. In this sense, when testing advances regarding aquaculture production, a sensory analysis is recommended, considering several aspects can influence the organoleptic properties of fish, including water temperature, rearing systems, sacrifice method and feeds used (Alak et al., 2024; Drake et al., 2006; Imsland et al., 2021; Lawley et al., 2012; Lyu et al., 2015; Ma et al., 2023; Pereira et al., 2022; Wang et al., 2024). Additionally, the use of natural and sustainable practices in aquaculture, with a focus on circular economy, and the reduction of the use of synthetic compounds including antibiotics, can also improve consumer's perception. That information should then be made accessible to consumers, so they can be aware that the aquaculture sector is investing in eco-friendlier and more sustainable practices. Finally, considering that animal welfare is becoming more and more significant for consumers, developing strategies that improve fish well-being in aquaculture is crucial.

Most of the aforementioned aspects can be addressed through dietary manipulation. Although consumers are not aware of it, aquafeeds are a very significant issue regarding aquaculture sustainability, influencing a fish farm's contribution to abiotic depletion, global warming, marine pollution, acidification, eutrophication and energy demands, as observed from several life cycle analysis (Ayer & Tyedmers, 2009; Bohnes et al., 2019; Ghamkhar et al., 2021). In this sense, the strategies developed in chapters 3 and 4 could ease consumers worries regarding aquaculture products and increase their acceptance. Plant-based aquafeeds could be perceived as being more natural, and their supplementation with ingredients obtained from by-products of other industries could allow consumers to perceive aquaculture products as more environmentally friendly and sustainable. Additionally, the hydrolysate RNF-H, as shown in chapter 3.1, can be used to enhance resistance to a pathogen, minimizing the need for antibiotics (chemicals), which is one of the main concerns voiced by consumers regarding farmed fish. Moreover, in chapter 4.2., we have shown that aquaculture products can be positively evaluated by consumers regarding their organoleptic characteristics. Overall, we believe that the developed aquafeed strategies could improve aquaculture perception if adequately disseminated. It is necessary to promote the increase of knowledge related to aquaculture among the general consumers, in order for these strategies to actually affect consumer perception. Campaigns on social media and television, coupled with the dissemination of the latest advances in aquaculture through public events, can indeed play a vital role in addressing consumer misinformation and fostering a more positive perception of aquaculture.

6.4. Main conclusions

This thesis aims to promote sustainable growth in aquaculture by understanding consumer preferences, minimizing economic and environmental impacts through the use of sustainable ingredients in fish diets, particularly those sourced from agri-food by-products, and enhancing consumer acceptance of farmed fish through strategic improvements. An overall scheme of this thesis and main conclusions can be found in Figure 3.

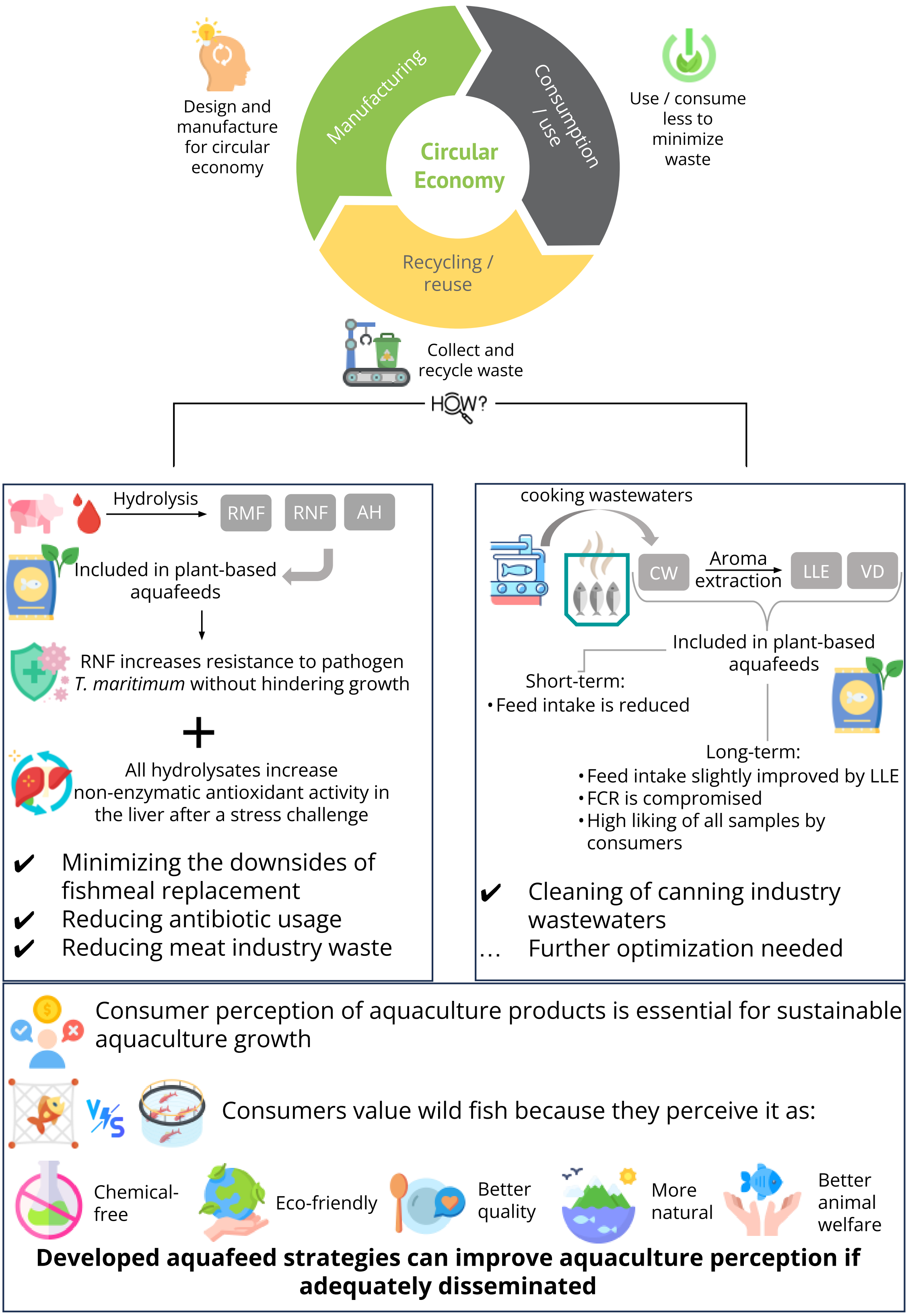

Figure 3 – Overall scheme of this thesis.

Based on the obtained results, the following conclusions can be drawn:

- Agri-food by-products can be valorised through processing to obtain ingredients for use in aquafeeds.
- Hydrolysates from swine blood were produced, using either an autohydrolysis or enzymatic hydrolysis approach.
- The production method and post-hydrolysis processing of hydrolysates affect their bioactive properties.
- One fraction, produced enzymatically and fractioned through membrane filtration (RNF-H), improved European seabass resistance to an infectious agent (*Tenacibaculum maritimum*) without compromising fish growth, when included in a plant-based aquafeed.
- The developed hydrolysates had minimal impact on the European seabass oxidative stress response, despite an increase in non-enzymatic antioxidant activity in the liver.
- Extracts from sardine cooking waters, rich in aroma compounds, were obtained and incorporated in plant-based aquafeeds.
- The extracts had a negative impact at the short-term regulation of feed intake, possible due to a hedonic regulation of feed intake, but that was not reflected at a longer term.
- One of the developed extracts, obtained through liquid-liquid extraction with soybean oil, increased feed intake, possibly due to a homeostatic regulation of intake.
- Plant-based diets, supplemented or not with the aromas, resulted in fish fillets with similar sensory profiles and well accepted by consumers.
- There is a very low objective and subjective knowledge from consumers regarding the differences between farmed and wild fish products.
- Generally, wild fish is perceived by consumers worldwide as having a better quality, possessing lower quantities of chemicals, being more natural and being environmentally friendlier than farmed fish.
- Consumers value not only high-quality products, but also products that have ethical attributes, such as promoting sustainability or animal welfare.
- The developed aquafeed supplementation strategies could improve aquaculture perception if adequately disseminated.

6.5. Future perspectives

In this study, we aimed to develop sustainable aquafeeds by adopting a circular economy approach, which involved supplementing plant-based diets with functional additives derived from agrifood by-products. This strategy aligns with the principles of sustainability by repurposing waste materials and maximizing resource efficiency in aquafeed production. Two types of additives were developed: hydrolysates and aroma extracts.

In the future, the impact of the dietary inclusion of the developed hydrolysates, particularly RNF-H (which displayed the strongest benefits, namely regarding improved immunity), should be further explored. In particular, the mechanisms behind the increase in the resistance to the pathogen *T. maritimum* should be better understood. For example, a study involving the sampling of infected fish could be conducted, analysing several biomarkers not only in the plasma, but also in the head kidney of the fish, which plays a key role in initiation of the immune response. After fully understanding these mechanisms, the potential of this hydrolysate to improve resistance to other pathogenic agents that affect European seabass could be tested. The study of the dietary inclusion of the hydrolysate for other fish species is also recommended. Furthermore, the hydrolysate obtained by autohydrolysis (AH-H) could potentially benefit from additional fractionation, similar to the process applied for enzymatic hydrolysates. This avenue warrants exploration to optimize the utilization and efficacy of AH-H in aquafeed formulations.

One of the difficulties associated with the trial performed for assessment of the aroma extracts was the lack of available knowledge regarding the response of European seabass to several volatile compounds. Therefore, the response of this species to compounds such as alcohols, aldehydes, ketones and organic acids should be thoroughly assessed. With that information, the extracts could be optimized to maximize the concentration of the compounds that promote the strongest response in European seabass. Studies with several dosages of the aromas should also be performed, as to optimize the inclusion levels of these extracts. Furthermore, the developed extracts, while not being particularly beneficial for European seabass, can still prove useful as attractants for other species of fish or even other animal models, such as cats, and this should be further explored.

Regarding the consumer perception of farmed fish, a deeper and updated study regarding Portuguese consumer's perception of aquaculture products should be conducted. The importance of several dimensions, including health, nutritional properties, sensory appeal, natural content, price and ethical concerns, in the purchase of farmed fish by Portuguese consumers, should be evaluated, as they may differ from others with a different cultural, social or economic background. Finally, considering that this work revealed a low knowledge from the consumer regarding aquaculture, which affects its perception, increasing consumers' literacy in this matter is essential. Dissemination of information regarding aquaculture practices, its efforts to minimize environmental impacts and increase fish welfare, and the lack of antibiotic residues on fish products should be promoted to improve perception of aquaculture products.

References

A. Costantino. (2023). *Our Products*. Retrieved on 16-02-2024 from https://www.acostantino.com/products.html

Alak, G., Kara, A., Akköse, A., Gelen, S. U., Tanas, Ş. T., Uçar, A., Parlak, V., & Atamanalp, M. (2024). Effect of climate change on fillet quality and shelf-life of *Oncorhynchus mykiss* under controlled conditions. *Journal of the Science of Food and Agriculture*, *104*(3), 1511-1520. https://doi.org/10.1002/jsfa.13034

Albendea, P., Tres, A., Rafecas, M., Vichi, S., Sala, R., & Guardiola, F. (2023). Effect of feeding acid oils on European seabass fillet lipid composition, oxidative stability, color, and sensory acceptance. *Aquaculture Nutrition*, *2023*, 6415693. https://doi.org/10.1155/2023/6415693

Amirkolaie, A. K. (2011). Reduction in the environmental impact of waste discharged by fish farms through feed and feeding. *Reviews in Aquaculture*, *3*(1), 19-26. https://doi.org/10.1111/j.1753-5131.2010.01040.x

Ankamah-Yeboah, I., Jacobsen, J. B., & Olsen, S. B. (2018). Innovating out of the fishmeal trap: The role of insect-based fish feed in consumers' preferences for fish attributes. *British Food Journal*, *120*(10), 2395-2410. https://doi.org/10.1108/BFJ-11-2017-0604

APC. (2024). *Our Ingredients*. Retrieved on 02-02-2024 from https://apcpet.com/our-products

Araújo-Rodrigues, H., Coscueta, E. R., Pereira, M. F., Cunha, S. A., Almeida, A., Rosa, A., Martins, R., Pereira, C. D., & Pintado, M. E. (2022). Membrane fractionation of *Cynara cardunculus* swine blood hydrolysate: ingredients of high nutritional and nutraceutical value. *Food Research International*, *158*, 111549. https://doi.org/http://doi.org/10.1016/j.foodres.2022.111549

Ares, G., Bruzzone, F., Vidal, L., Cadena, R. S., Giménez, A., Pineau, B., Hunter, D. C., Paisley, A. G., & Jaeger, S. R. (2014). Evaluation of a rating-based variant of check-all-that-apply questions: Rate-all-that-apply (RATA). *Food Quality and Preference*, *36*, 87-95. https://doi.org/10.1016/j.foodqual.2014.03.006

Arsel, Z. (2017). Asking questions with reflexive focus: A tutorial on designing and conducting interviews. *Journal of Consumer Research*, *44*(4), 939-948. https://doi.org/10.1093/jcr/ucx096

Avendaño-Herrera, R., Toranzo, A., & Magariños, B. (2006). Tenacibaculosis infection in marine fish caused by *Tenacibaculum maritimum*: a review. *Diseases of Aquatic Organisms*, *71*, 255-266. https://doi.org/10.3354/dao071255

Ayer, N. W., & Tyedmers, P. H. (2009). Assessing alternative aquaculture technologies: life cycle assessment of salmonid culture systems in Canada. *Journal of Cleaner Production*, *17*(3), 362-373. https://doi.org/https://doi.org/10.1016/j.jclepro.2008.08.002

Baek, S. I., Cho, S. H., & Kim, H. S. (2021). Dietary inclusion effect of various levels of jack mackerel meal on the growth performance, feed efficiency and whole body composition of rockfish (*Sebastes schlegeli*). *Fisheries and Aquatic Sciences*, *24*(9), 311-317. https://doi.org/10.47853/FAS.2021.e30

Barroso, F. G., Rodiles, A., Vizcaino, A. J., Martínez, T. F., & Alarcón, F. J. (2013). Evaluation of feed attractants in juvenile Senegalese sole, *Solea senegalensis*. *Journal of the World Aquaculture Society*, *44*(5), 682-693. https://doi.org/10.1111/jwas.12068

Bohnes, F. A., Hauschild, M. Z., Schlundt, J., & Laurent, A. (2019). Life cycle assessments of aquaculture systems: a critical review of reported findings with recommendations for policy and system development. *Reviews in Aquaculture*, *11*(4), 1061-1079. https://doi.org/https://doi.org/10.1111/raq.12280

Calo, J., Comesaña, S., Fernández-Maestú, C., Blanco, A. M., Morais, S., & Soengas, J. L. (2024). Impact of feeding diets with enhanced vegetable protein content and presence of umami taste-stimulating additive on gastrointestinal amino acid sensing and feed intake regulation in rainbow trout. *Aquaculture*, *579*, 740251. https://doi.org/https://doi.org/10.1016/j.aquaculture.2023.740251

Camacho-Luna, P., Andrews, F. M., Keowen, M. L., Garza Jr, F., Liu, C.-C., Lamp, B., & Olijve, J. (2022). The effect of porcine hydrolysed collagen on gastric ulcer scores, gastric juice pH, gastrin and amino acid concentrations in horses. *Equine Veterinary Education*, *34*(5), 248-257. https://doi.org/10.1111/eve.13409

Cantillo, J., Martin, J. C., & Roman, C. (2020). Discrete choice experiments in the analysis of consumers' preferences for finfish products: A systematic literature review. *Food Quality and Preference*, *84*, 103952, Article 103952. https://doi.org/10.1016/j.foodqual.2020.103952

Cao, T. N.-D., Bui, X.-T., Le, L.-T., Dang, B.-T., Tran, D. P.-H., Vo, T.-K.-Q., Tran, H.-T., Nguyen, T.-B., Mukhtar, H., Pan, S.-Y., Varjani, S., Ngo, H. H., & Vo, T.-D.-H. (2022). An overview of deploying membrane bioreactors in saline wastewater treatment from perspectives of microbial and treatment performance. *Bioresource Technology*, *363*, 127831. https://doi.org/10.1016/j.biortech.2022.127831

Comesaña, S., Velasco, C., Ceinos, R. M., López-Patiño, M. A., Míguez, J. M., Morais, S., & Soengas, J. L. (2018). Evidence for the presence in rainbow trout brain of amino acid-sensing systems involved in the control of food intake. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *314*(2), R201-R215. https://doi.org/10.1152/ajpregu.00283.2017

Corassa, A., Lopes, D. C., Pena, S. d. M., Freitas, L. S. d., & Pena, G. d. M. (2007). Hydrolyzed of intestinal mucosa of swine in substitution to blood plasma in diets of pigs from 21 to 49 days. *Revista Brasileira de Zootecnia*, *36*. https://doi.org/10.1590/S1516-35982007000900012

Cros, S., Ignot, B. L., Razafintsalama, C., Jaouen, P., & Ourseau, P. B. (2004). Electrodialysis desalination and reverse osmosis concentration of an industrial mussel cooking juice: Process impact on pollution reduction and on aroma quality. *Journal of Food Science*, *69*(6), C435-C442. https://doi.org/10.1111/j.1365-2621.2004.tb10985.x

Dias, J., Gomes, E. F., & Kaushik, S. (1997). Improvement of feed intake through supplementation with an attractant mix in European bass fed plant-protein rich diets. *Aquatic Living Resources*, *10*. https://doi.org/10.1051/alr:1997043

dos Santos Cardoso, M., Godoy, A. C., Oxford, J. H., Rodrigues, R., dos Santos Cardoso, M., Bittencourt, F., Signor, A., Boscolo, W. R., & Feiden, A. (2021). Apparent digestibility of protein hydrolysates from chicken and swine slaughter residues for Nile tilapia. *Aquaculture*, *530*, 735720. https://doi.org/10.1016/j.aquaculture.2020.735720

Drake, S. L., Drake, M. A., Daniels, H. V., & Yates, M. D. (2006). Sensory properties of wild and aquacultured southern flounder (*Paralichthys lethostigma*). *Journal of Sensory Studies*, *21*(2), 218-227. https://doi.org/10.1111/j.1745-459X.2006.00062.x

Dumas, A., de Lange, C. F. M., France, J., & Bureau, D. P. (2007). Quantitative description of body composition and rates of nutrient deposition in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *273*(1), 165-181. https://doi.org/10.1016/j.aquaculture.2007.09.026

Fernández-Alacid, L., Firmino, J. P., Sanahuja, I., Madrid, C., Polo, J., de Borba, M. R., Balsalobre, C., Gisbert, E., & Ibarz, A. (2021). Impact of dietary porcine blood by-products in meagre (*Argyrosomus regius*) physiology, evaluated by welfare biomarkers and the antibacterial properties of the skin mucus. *Fish & Shellfish Immunology*, *118*, 241-250. https://doi.org/10.1016/j.fsi.2021.09.011

Fernández-Alacid, L., Sanahuja, I., Madrid, C., Polo, J., Firmino, J. P., Balsalobre, C., Reyes-López, F. E., Vallejos-Vidal, E., Andree, K. B., Gisbert, E., & Ibarz, A. (2022). Evaluating the functional properties of spray-dried porcine plasma in gilthead seabream (*Sparus aurata*) fed low fish meal diets. *Animals*, *12*(23), 3297. https://doi.org/10.3390/ani12233297

Ferraro, V., Carvalho, A. P., Piccirillo, C., Santos, M. M., L. Castro, P. M., & E. Pintado, M. (2013). Extraction of high added value biological compounds from sardine, sardine-type fish and mackerel canning residues — A review. *Materials Science and Engineering: C*, *33*(6), 3111-3120. https://doi.org/10.1016/j.msec.2013.04.003

Ghamkhar, R., Boxman, S. E., Main, K. L., Zhang, Q., Trotz, M. A., & Hicks, A. (2021). Life cycle assessment of aquaculture systems: Does burden shifting occur with an increase in production intensity? *Aquacultural Engineering*, *92*, 102130. https://doi.org/https://doi.org/10.1016/j.aquaeng.2020.102130

Giri, A., Osako, K., & Ohshima, T. (2010). Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. *Food Chemistry*, *120*(2), 621-631. https://doi.org/10.1016/j.foodchem.2009.10.036

Gisbert, E., Ibarz, A., Firmino, J. P., Fernández-Alacid, L., Salomón, R., Vallejos-Vidal, E., Ruiz, A., Polo, J., Sanahuja, I., Reyes-López, F. E., Tort, L., & Andree, K. B. (2021). Porcine protein hydrolysates (PEPTEIVA®) promote growth and enhance systemic immunity in gilthead sea bream (*Sparus aurata*). *Animals*, *11*(7), 2122. https://doi.org/10.3390/ani11072122

Gisbert, E., Skalli, A., Fernández, I., Kotzamanis, Y., Zambonino-Infante, J. L., & Fabregat, R. (2012). Protein hydrolysates from yeast and pig blood as alternative raw materials in microdiets for gilthead sea bream (*Sparus aurata*) larvae. *Aquaculture*, *338-341*, 96-104. https://doi.org/https://doi.org/10.1016/j.aquaculture.2012.01.007

Grimsrud, K. M., Nielsen, H. M., Navrud, S., & Olesen, I. (2013). Households' willingness-to-pay for improved fish welfare in breeding programs for farmed Atlantic salmon. *Aquaculture*, *372*, 19-27. https://doi.org/10.1016/j.aquaculture.2012.10.009

Hansen, H., & Onozaka, Y. (2011). When diseases hit aquaculture: An experimental study of spillover effects from negative publicity. *Marine Resource Economics*, *26*(4), 281-291. https://doi.org/10.5950/0738-1360-26.4.281

Hoerterer, C., Petereit, J., & Krause, G. (2022). Informed choice: The role of knowledge in the willingness to consume aquaculture products of different groups in Germany. *Aquaculture*, *556*, 738319. https://doi.org/10.1016/j.aquaculture.2022.738319

Hu, X., Liu, Y., Zhao, Z., Liu, J., Yang, X., Sun, C., Chen, S., Li, B., & Zhou, C. (2021). Real-time detection of uneaten feed pellets in underwater images for aquaculture using an improved YOLO-V4 network. *Computers and Electronics in Agriculture*, *185*, 106135. https://doi.org/10.1016/j.compag.2021.106135

Imsland, A. K. D., Ólafsdóttir, A., Árnason, J., Gústavsson, A., Thorarensen, H., & Gunnarsson, S. (2021). Effect of rearing temperature on flesh quality in Arctic charr (*Salvelinus alpinus*). *Aquaculture Research*, *52*(3), 1063-1070. https://doi.org/10.1111/are.14961

Izquierdo, M. S., Montero, D., Robaina, L., Caballero, M. J., Rosenlund, G., & Ginés, R. (2005). Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. *Aquaculture*, *250*(1), 431-444. https://doi.org/10.1016/j.aquaculture.2004.12.001

Izquierdo, M. S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L., & Rosenlund, G. (2003). Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. *Aquaculture Nutrition*, *9*(6), 397-407. https://doi.org/10.1046/j.1365-2095.2003.00270.x

Jamroz, D., Wiliczkiewicz, A., Orda, J., Skorupińska, J., Słupczyńska, M., & Kuryszko, J. (2011). Chemical composition and biological value of spray dried porcine blood by-products and bone protein hydrolysate for young chickens. *British Poultry Science*, *52*(5), 589-605. https://doi.org/10.1080/00071668.2011.610298

Jarrault, C., Dornier, M., Labatut, M.-L., Giampaoli, P., & Lameloise, M.-L. (2017). Coupling nanofiltration and osmotic evaporation for the recovery of a natural flavouring concentrate from shrimp cooking juice. *Innovative Food Science & Emerging Technologies*, *43*, 182-190. https://doi.org/10.1016/j.ifset.2017.07.035

Jeong, H. S., Choi, D. G., Lee, K. W., Cho, S. H., Lim, S. G., Lee, B. J., Hur, S. W., Son, M. H., Lee, S. H., & Kim, K. W. (2020). Attractiveness of various crude feed ingredients to juvenile olive flounder (*Paralichthys olivaceus*, Temminck & Schlegel) and its application to aquaculture. *Aquaculture Research*, *51*(11), 4517-4532. https://doi.org/10.1111/are.14797

Jin, S. W., Lee, G. H., Kim, J. Y., Kim, C. Y., Choo, Y. M., Cho, W., Han, E. H., Hwang, Y. P., Kim, Y. A., & Jeong, H. G. (2022). Effect of Porcine Whole Blood Protein Hydrolysate on Slow-Twitch Muscle Fiber Expression and Mitochondrial Biogenesis via the AMPK/SIRT1 Pathway. *International Journal of Molecular Sciences*, *23*(3), 1229. https://doi.org/10.3390/ijms23031229

Kasumyan, A. O., & Døving, K. B. (2003). Taste preferences in fishes. *Fish and Fisheries*, *4*(4), 289-347. https://doi.org/10.1046/j.1467-2979.2003.00121.x

Khalil, R. H., Diab, A. M., Shakweer, M. S., Ghetas, H. A., Khallaf, M. M., & Omar, A. (2018). New perspective to control of tenacibaculosis in sea bass *Dicentrarchus labrax* L. *Aquaculture Research*, *49*(7), 2357-2365. https://doi.org/10.1111/are.13689

Lawley, M., Birch, D., & Hamblin, D. (2012). An exploratory study into the role and interplay of intrinsic and extrinsic cues in Australian consumers' evaluations of fish. *Australasian Marketing Journal*, *20*(4), 260-267. https://doi.org/10.1016/j.ausmj.2012.05.014

Liang, X., Han, J., Xue, M., Yu, H., Huang, H., Wu, X., Zheng, Y., Qin, Y., & Liang, X. (2019). Growth and feed intake regulation responses to anorexia, adaptation and fasting in Japanese seabss, *Lateolabrax japonicas* when fishmeal is totally replaced by plant protein. *Aquaculture*, *498*, 528-538. https://doi.org/10.1016/j.aquaculture.2018.09.010

Lizin, S., Rousseau, S., Kessels, R., Meulders, M., Pepermans, G., Speelman, S., Vandebroek, M., Van Den Broeck, G., Van Loo, E. J., & Verbeke, W. (2022). The state of the art of discrete choice experiments in food research. *Food Quality and Preference*, *102*, 104678. https://doi.org/10.1016/j.foodqual.2022.104678

Lyu, F., Huang, R.-J., Liu, L., Zhou, X., & Ding, Y.-T. (2015). Effect of slaughter methods on the quality of Chilean jack mackerel (Trachurus murphyi) during refrigerated storage. *Journal of Food Science and Technology*, *52*(3), 1742-1747. https://doi.org/10.1007/s13197-013-1114-8

Ma, Y.-B., Zou, W.-G., Ai, C.-X., Luo, X., Liu, S.-T., Huang, H.-Q., Chen, Y.-X., You, W.-W., & Ke, C.-H. (2023). Interactive effects of diet and water temperature on the nutritional value, flavor, and sensory quality in hybrid abalone (*Haliotis. discus hannai* ♀ × *H. fulgens*♂) meat. *Aquaculture*, *567*, 739241. https://doi.org/10.1016/j.aquaculture.2023.739241

Mabrok, M., Algammal, A. M., Sivaramasamy, E., Hetta, H. F., Atwah, B., Alghamdi, S., Fawzy, A., Avendaño-Herrera, R., & Rodkhum, C. (2023). Tenacibaculosis caused by *Tenacibaculum maritimum*: Updated knowledge of this marine bacterial fish pathogen. *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/fcimb.2022.1068000

Marfà , O., Cáceres, R., Polo, J., & Ródenas, J. (2009). Animal protein hydrolysate as a biostimulant for transplanted strawberry plants subjected to cold stress. *Acta Horticulturae*, *842*, 315-318. https://doi.org/10.17660/ActaHortic.2009.842.57

Márquez, E., Bracho, M., Archile, A., Rangel, L., & Benítez, B. (2005). Proteins, isoleucine, lysine and methionine content of bovine, porcine and poultry blood and their fractions. *Food Chemistry*, *93*(3), 503-505. https://doi.org/10.1016/j.foodchem.2004.10.030

Martínez, R., Sanz, M. T., & Beltrán, S. (2014). Pervaporation investigation of recovery of volatile compounds from brown crab boiling juice. *Food Science and Technology International*, *20*(7), 511-526. https://doi.org/10.1177/1082013213496092

Mateos, G. G., Mohiti-Asli, M., Borda, E., Mirzaie, S., & Frikha, M. (2014). Effect of inclusion of porcine mucosa hydrolysate in diets varying in lysine content on growth performance and ileal histomorphology of broilers. *Animal Feed Science and Technology*, *187*, 53-60. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2013.09.013

Matos, E., Gonçalves, A., Bandarra, N., Colen, R., Nunes, M. L., Valente, L. M. P., Dinis, M. T., & Dias, J. (2012). Plant proteins and vegetable oil do not have detrimental effects on post-mortem muscle instrumental texture, sensory properties and nutritional value of gilthead seabream. *Aquaculture*, *358-359*, 205-212. https://doi.org/10.1016/j.aquaculture.2012.07.009

Middelkoop, A., Segarra, S., & Molist, F. (2023). Porcine digestible peptides as alternative protein source in weaner diets: effects on performance and systemic cytokine profile in pigs followed from weaning to slaughter. *animal*, *17*(11), 100998. https://doi.org/10.1016/j.animal.2023.100998

MobFood Consortium. (2017). *About MobFood*. Retrieved on 29/03/2023 from https://mobfood.pt/en/project/

Novriadi, R., Spangler, E., Rhodes, M., Hanson, T., & Allen Davis, D. (2017). Effects of various levels of squid hydrolysate and squid meal supplementation with enzyme-treated soy on growth performance, body composition, serum biochemistry and histology of Florida pompano *Trachinotus carolinus*. *Aquaculture*, *481*, 85-93. https://doi.org/10.1016/j.aquaculture.2017.08.032

Olesen, I., Alfnes, F., Røra, M. B., & Kolstad, K. (2010). Eliciting consumers' willingness to pay for organic and welfare-labelled salmon in a non-hypothetical choice experiment. *Livestock Science*, *127*(2-3), 218-226. https://doi.org/10.1016/j.livsci.2009.10.001

Onwuegbuzie, A. J., Dickinson, W. B., Leech, N. L., & Zoran, A. G. (2009). A qualitative framework for collecting and analyzing data in focus group research. *International Journal of Qualitative Methods*, *8*(3), 1-21. https://doi.org/10.1177/160940690900800301

Pereira, R., Costa, M., Velasco, C., Cunha, L. M., Lima, R. C., Baião, L. F., Batista, S., Marques, A., Sá, T., Campos, D. A., Pereira, M., Jesus, D., Fernández-Boo, S., Costas, B., Pintado, M., & Valente, L. M. P. (2022). Comparative analysis between synthetic vitamin E and natural antioxidant sources from tomato, carrot and coriander in diets for market-sized *Dicentrarchus labrax*. *Antioxidants*, *11*(4), 636. https://doi.org/10.3390/antiox11040636

Petereit, J., Hoerterer, C., & Krause, G. (2022). Country-specific food culture and scientific knowledge transfer events – Do they influence the purchasing behaviour of seafood products? *Aquaculture*, *560*, 738590, Article 738590. https://doi.org/10.1016/j.aquaculture.2022.738590

Pintado, M., Araújo-Rodrigues, H., Pereira, M. F., Costas, B., Machado, M., Pereira, C. D., Valente, L. M. P., & Resende, D. (2022). *New Hydrolysates to Control Infections, Methods and Uses Thereof - International Patent Application Published under the Patent Cooperation Treaty.* WIPO | PCT.

Pleić, I. L., Bušelić, I., Messina, M., Hrabar, J., Žuvić, L., Talijančić, I., Žužul, I., Pavelin, T., Anđelić, I., Pleadin, J., Puizina, J., Grubišić, L., Tibaldi, E., & Šegvić-Bubić, T. (2022). A plant-based diet supplemented with *Hermetia illucens* alone or in combination with poultry by-product meal: one step closer to sustainable aquafeeds for European seabass. *Journal of Animal Science and Biotechnology*, *13*(1), 77. https://doi.org/10.1186/s40104-022-00725-z

Polo, J., Barroso, R. N., RóDenas, J. S., AzcóN-Bieto, J. N., CáCeres, R., & Marfà, O. (2006). Porcine hemoglobin hydrolysate as a biostimulant for lettuce plants subjected to conditions of thermal stress. *HortTechnology*, *16*(3), 483-487. https://doi.org/10.21273/horttech.16.3.0483

Risius, A., Janssen, M., & Hamm, U. (2017). Consumer preferences for sustainable aquaculture products: Evidence from in-depth interviews, think aloud protocols and choice experiments. *Appetite*, *113*, 246-254. https://doi.org/10.1016/j.appet.2017.02.021

Sabioni, R. E., Lorenz, E. K., Cyrino, J. E. P., & Volkoff, H. (2022). Feed intake and gene expression of appetite-regulating hormones in *Salminus brasiliensis* fed diets containing soy protein concentrate. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *268*, 111208. https://doi.org/10.1016/j.cbpa.2022.111208

Sandbakken, I. S., Five, K. K., Bardal, T., Knapp, J. L., & Olsen, R. E. (2023). Salmon hydrolysate as a protein source for Atlantic salmon; prion content and effects on growth, digestibility and gut health. *Aquaculture*, *576*, 739863. https://doi.org/10.1016/j.aquaculture.2023.739863

Soares, L. S., Vieira, A. C. F., Fidler, F., Fritz, A. R. M., & Di Luccio, M. (2020). Pervaporation as an alternative for adding value to residues of oyster (*Crassostrea gigas*) processing. *Separation and Purification Technology*, *232*, 115968. https://doi.org/10.1016/j.seppur.2019.115968

Solà-Oriol, D., Roura, E., & Torrallardona, D. (2011). Feed preference in pigs: Effect of selected protein, fat, and fiber sources at different inclusion rates1. *Journal of animal science*, *89*(10), 3219-3227. https://doi.org/10.2527/jas.2011-3885

Sonac. (2024a). *Innomax MPI*. Retrieved on 16-02-2024 from https://www.sonac.biz/where-worlds-meet/petfood-ingredients/bio-functional-ingredients/innomax-mpi

Sonac. (2024b). *MucoPro 80P: Pure guts power Pamphlet*. https://www.sonac.biz/ingredients/feed-pet-food-proteins-fats

Sonac. (2024c). *Palapro 80P Pamphlet*. https://www.sonac.biz/ingredients/blood-products

Sorapukdee, S., & Narunatsopanon, S. (2017). Comparative study on compositions and functional properties of porcine, chicken and duck blood. *Food Science of Animal Resources*, *37*(2), 228-241. https://doi.org/10.5851/kosfa.2017.37.2.228

Stewart, D. W., Shamdasani, P. N., & Rook, D. W. (2007). Focus Groups and the Research Toolbox. In *Focus Groups. Theory and Practice* (2nd ed., pp. 37-50). Sage Publications.

Thelwall, M., & Nevill, T. (2021). Is research with qualitative data more prevalent and impactful now? Interviews, case studies, focus groups and ethnographies. *Library & Information Science Research*, *43*(2), 101094. https://doi.org/10.1016/j.lisr.2021.101094

Torstensen, B. E., Espe, M., Sanden, M., Stubhaug, I., Waagbø, R., Hemre, G. I., Fontanillas, R., Nordgarden, U., Hevrøy, E. M., Olsvik, P., & Berntssen, M. H. G. (2008). Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. *Aquaculture*, *285*(1), 193-200. https://doi.org/10.1016/j.aquaculture.2008.08.025

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

Venkateshwarlu, G., Let, M. B., Meyer, A. S., & Jacobsen, C. (2004). Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. *Journal of Agricultural and Food Chemistry*, *52*(2), 311-317. https://doi.org/10.1021/jf034833v

Venugopal, V., & Sasidharan, A. (2021). Seafood industry effluents: Environmental hazards, treatment and resource recovery. *Journal of Environmental Chemical Engineering*, *9*(2), 104758. https://doi.org/10.1016/j.jece.2020.104758

Wang, Z., Qiao, F., Zhang, W.-B., Parisi, G., Du, Z.-Y., & Zhang, M.-L. (2024). The flesh texture of teleost fish: Characteristics and interventional strategies. *Reviews in Aquaculture*, *16*(1), 508-535. https://doi.org/10.1111/raq.12849

Williamson, C. (2013). Questionnaires, individual interviews and focus groups. In K. Williamson & G. Johanson (Eds.), *Research Methods: Information, Systems, and Contexts* (pp. 349-372). Tilde University Press.

Yang, X., Wang, G., Zhao, X., Dong, X., Chi, S., & Tan, B. (2021). Addition of hydrolysed porcine mucosa to low-fishmeal feed improves intestinal morphology and the expressions of intestinal amino acids and small peptide transporters in hybrid groupers (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂). *Aquaculture*, *535*, 736389. https://doi.org/10.1016/j.aquaculture.2021.736389

Zhang, Y., Zhang, Y., Ke, T., Shi, B., Huang, L., Dong, Z., Guo, M., Mugeni, C. S., Zhu, A., Wang, L., Xu, J., & Storebakken, T. (2022). Hydrolysates of whole forage-fish and Pacific krill are useful to reduce fish meal in practical diets for largemouth bass (*Micropterus salmoides*), and dietary fish hydrolysate suppresses expressions of intestinal oligopeptide transporter and taurine transporter genes. *Aquaculture Reports*, *25*, 101203. https://doi.org/10.1016/j.aqrep.2022.101203

"Your world is nothing more than all the tiny things you've left behind."

- Jamie Cullum, *Gran Torino*

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