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Fachbereich 2

Effects of alternative feed ingredients on the growth of *Dicentrarchus labrax* and the circadian rhythm of *Scophthalmus maximus* in recirculating aquaculture systems linked to consumer perception of seafood in Europe

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You may encounter many defeats, but you must not be defeated. In fact, it may be necessary to encounter the defeats, so you can know who you are, what you can rise from, how you can still come out of it.
- Maya Angelou

Contents

Abbreviations

AA	Amino Acid
ANOVA	Analysis of variance
ASC	Aquaculture Stewardship Council
AWI	Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research
CE	Circular Economy
CR	Circadian Rhythm
EAA	Essential Amino Acid
EC	European Commission
EU	European Union
EAA	European Economic Area
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
FPH	Fish Protein Hydrolysates
GAIN	Green Aquaculture Intensification in Europe
I	Iod
LCA	Life Cycle Assessment
mmt	Million Metric Tons
NMR	Nuclear Magnetic Resonance
PER	Protein Efficiency Ratio
RAS	Recirculating Aquaculture System
SBM	Soybeanmeal
SDG	Sustainable Development Goals
Se	Selen
SHP	Salten Havbrukspark AS
UN	United Nations
VPC	Vegetable Protein Concentrate
WU	Wageningen University
YM	Yeast Meal

Summary

This PhD thesis recommends increasing sustainable aquaculture consumption and production in Europe. The research is based on a interdisciplinary approach combining European consumer perspectives with new feed concepts for European seabass (*Dicentrarchus labrax*) and detecting the circadian rhythm in turbot (*Scophthalmus maximus*) to improve welfare and develop potential new management strategies for aquaculture operations.

Aquaculture remains the fastest growing food production sector in the world, and sustainable systems are needed to ensure food security and achieve the Sustainable Development Goals (SDGs). A promising and widely used solution for the future of intensive fish farming is the combination of circular economy and recirculating aquaculture systems (RAS). This approach could increase productivity and reduce environmental impact and resource use, resulting in improved consumer perception. That is important since consumers are the driving force behind seafood consumption, and their purchasing behaviour shapes the appearance of sustainable fish products in the supermarket. Understanding the consumer perspective, therefore, represents the first step towards sustainable production. The findings of my PhD indicate that the most relevant factors for consumers to purchase sustainable aquaculture products are fish origin (including farming systems), sustainability, and fish welfare. Unfortunately, aquaculture seafood production continues to be considered negatively by the public since there have been many serious ecological and environmental problems in recent decades, such as the misuse of antibiotics. The major concerns are related to the proportion of wild-caught fish in aquaculture feeds and the welfare of farmed animals. Consequently, developing alternative feeds and improving fish welfare have the potential to shift consumer perceptions towards more positive attitudes.

Intensive fish farms could develop and implement new strategies to counteract those concerns. Product descriptions as well as eco-labels, can contribute to consumer awareness and could be considered with a life-cycle assessment (LCA), which could include carbon footprints and energy and water use. My findings suggest that an information-based strategy focussing on the product's origin and its sustainability could be an effective tool to change consumer awareness and encourage fish farmers to invest in sustainable feeds and farming techniques. LCAs have shown that unsustainably produced fish feeds greatly impact intensive aquaculture operations, such as RAS. Feed

for carnivorous fish often contains high levels of fishmeal and fishoil, which are often considered unsustainable resources. To that end, the second part of my PhD addresses alternative feed concepts for European seabass to improve sustainability while ensuring fish growth, farm profitability, and animal welfare. European seabass represents the most important aquaculture species in the Mediterranean Sea and ranks second only to salmon in the highest per capita consumption in the European Union. My work indicates that hydrolysates and aquaculture by-products, as well as plant- and animal-derived protein substitutions, can replace fishmeal from marine fish stocks without negative effects on growth, feed conversion ratio, sensory attributes, nutritional components, and fish welfare. This demonstrates that European seabass diets could become more sustainable through the application of circular economy approach and the use of plant and terrestrial animal-derived proteins.

In the last part of my PhD thesis, I examined the welfare of fish in greater depth. Fish and every other living organism possess a circadian rhythm (CR) that controls all metabolic processes. This CR is species-specific, and to my knowledge, no data are yet available for turbot. Turbot is a very valuable species in European aquaculture and understanding its day and night rhythms is necessary for improving its welfare and thus its growth in intensive aquaculture farms. My findings imply metabolic peaks in amino acids and sugars at 3 p.m., which could be used to optimise feeding regimes and farming practices. Specifically, on-farm activities, such as feeding (digestive stress) or transportation, can increase stress and should be avoided during peak levels of stress hormones. Utilising variations in natural metabolism combined with circular economy and optimised feeding concepts could help to improve animal welfare and growth.

This thesis provides a comprehensive picture of the impact of the different interdisciplinary approaches in European aquaculture, from the consumer to the individual fish. Implementing sustainable and regional ingredients in the diets of carnivorous fish can not only decrease the dependency on raw materials but also positively influence the consumer purchase decisions towards aquaculture. By suggesting optimised feeding times to improve fish welfare, my work provides ideas for novel farm practices that improve both farm profitability and consumer perception.

Zusammenfassung

In dieser Dissertation werden Empfehlungen zur Steigerung des nachhaltigen Verbrauchs und der Produktion in der europäischen Aquakultur gegeben. Die Forschung basiert auf einem transdisziplinären Ansatz, der europäische Verbraucherperspektiven mit neuen Futterkonzepten für den Europäischen Wolfsbarsch (*Dicentrarchus labrax*) und der Erkennung des zirkadianen Rhythmus beim Steinbutt (Scophthalmus maximus) kombiniert, um das Wohlergehen der Tiere zu verbessern und potenzielle neue Managementstrategien für Aquakulturbetriebe zu entwickeln.

Die Aquakultur ist nach wie vor der am schnellsten wachsende Sektor der Lebensmittelproduktion weltweit, und es werden nachhaltige Systeme benötigt, um die Ernährungssicherheit zu gewährleisten und die Ziele für nachhaltige Entwicklung (SDGs) zu erreichen. Eine vielversprechende und weit verbreitete Lösung für die Zukunft der intensiven Fischzucht ist die Kombination aus Kreislaufwirtschaft und rezirkulierenden Aquakultursystemen (RAS). Dieser Ansatz könnte die Produktivität erhöhen und die Umweltbelastung und den Ressourcenverbrauch verringern, was zu einer verbesserten Wahrnehmung durch die Verbraucher führt. Das ist wichtig, da die Verbraucher die treibende Kraft hinter dem Konsum von Meeresfrüchten sind, und ihr Kaufverhalten das Erscheinungsbild nachhaltiger Fischprodukte im Supermarkt beeinflusst. Das Verständnis der Verbraucherperspektive ist daher der erste Schritt zu einer nachhaltigeren Produktion. Die Ergebnisse meiner Doktorarbeit deuten darauf hin, dass die wichtigsten Faktoren für die Verbraucher, beim Kauf nachhaltiger Aquakulturprodukte, die Herkunft der Fische (einschließlich ihrer Hältungssysteme), die Nachhaltigkeit und das Wohlergehen der Tiere sind. Leider wird die Zucht von Fischen in intensiven Aquakulturen von der Öffentlichkeit nach wie vor negativ beurteilt, da es in den letzten Jahrzehnten viele schwerwiegende ökologische Probleme gegeben hat, wie

z. B. den Missbrauch von Antibiotika. Die größten Bedenken beziehen sich allerdings auf den Anteil von Wildfängen im Aquakulturfutter und das Wohlergehen der Zuchttiere. Die Entwicklung alternativer Futtermittel und die Verbesserung des Wohlergehens der Fische haben daher das Potenzial, die Wahrnehmung der Verbraucher in Richtung einer positiveren Einstellung zu verändern und somit das Kaufpotential zu steigern.

Durch die Entwicklung neuer Strategien könnten intensive diesen Bedenken entgegenwirken. Produktbeschreibungen und Öko-Labels könnten so zur Sensibilisierung der Verbraucher beitragen und in Verbindung mit einer

Lebenszyklusanalyse (LCA) oder auch Ökobilianz ergänzt werden. Diese könnte den Kohlenstoff-Fußabdruck sowie den Energie- und Wasserverbrauch miteinbeziehen und für mehr Transparenz für den Konsumenten sorgen. Meine Ergebnisse deuten darauf hin, dass eine informationsbasierte Strategie, die sich auf die Herkunft des Produktes sowie seine Nachhaltigkeit konzentriert, ein wirksames Instrument sein könnte, um das Bewusstsein der Verbraucher zu ändern als auch die Fischzüchter zu ermutigen, in nachhaltige Futtermittel und Zuchttechniken zu investieren. Ökobilianzen haben gezeigt, dass nicht nachhaltig produzierte Fischfuttermittel intensive Aquakulturbetriebe, wie z. B. RAS, stark negativ beeinträchtigen. Futtermittel für karnivore Fische enthalten oft einen hohen Anteil an Fischmehl und Fischöl, die oft als nicht nachhaltige Ressourcen angesehen werden. Aus diesem Grund befasst sich der zweite Teil meiner Doktorarbeit mit alternativen Futterkonzepten für Wolfsbarsch, um die Nachhaltigkeit zu verbessern und gleichzeitig das Fischwachstum, die Rentabilität der Zuchtbetriebe und das Wohlergehen der Tiere zu gewährleisten. Der Europäische Wolfsbarsch ist die wichtigste Aquakulturart im Mittelmeer und steht nach dem Lachs an zweiter Stelle des höchsten Pro-Kopf-Verbrauchs in der Europäischen Union. Meine Arbeit zeigt, dass Hydrolysate und Nebenprodukte aus der Aquakultur sowie pflanzliche und tierische Proteine Fischmehl aus marinen Fischbeständen ersetzen können, ohne dass dies negative Auswirkungen auf das Wachstum, die Futterverwertung, die sensorischen Eigenschaften, die Ernährungskomponenten oder das Wohlergehen der Fische hat. Dies zeigt, dass die Futtermittel des europäischen Wolfsbarsches durch die Anwendung einer Kreislaufwirtschaft und die Verwendung von pflanzlichen und tierischen Proteinen nachhaltiger werden kann.

Im letzten Teil meiner Doktorarbeit habe ich das Wohlergehen von Fischen eingehender untersucht. Fische und alle anderen Lebewesen verfügen über einen zirkadianen Rhythmus (CR), der alle Stoffwechselprozesse steuert. Dieser CR ist artspezifisch, und meines Wissens liegen für Steinbutt noch keine Daten vor. Der Steinbutt ist eine sehr wertvolle Art in der europäischen Aquakultur, und das Verständnis seiner Tag- und Nachtrhythmen ist notwendig, um sein Wohlergehen und damit sein Wachstum in intensiven Aquakulturbetrieben zu verbessern. Meine Ergebnisse deuten auf Stoffwechselspitzen bei Aminosäuren und Zuckern um 15 Uhr hin, die zur Optimierung von Fütterungsregimen und Zuchtpraktiken genutzt werden könnten. Insbesondere können Tätigkeiten im Betrieb, wie Fütterung (welcher Verdauungsstress auslösen kann) oder Transport, den Stress erhöhen und sollten während der Spitzenwerte von Stress Metaboliten vermieden werden. Die Nutzung von Schwankungen des natürlichen

Stoffwechsels in Kombination mit Kreislaufwirtschaft und optimierten Fütterungskonzepten könnte dazu beitragen, das Wohlergehen und das Wachstum der Tiere nachhaltig zu verbessern.

Diese Arbeit vermittelt ein umfassendes Bild der Auswirkungen der verschiedenen interdisziplinären Ansätze in der europäischen Aquakultur, vom Verbraucher bis zum einzelnen Fisch. Die Verwendung nachhaltiger und regionaler Futterstoffe in der Ernährung karnivorer Fische kann nicht nur die Abhängigkeit von Rohstoffen verringern, sondern auch die Kaufbereitschaft der Verbraucher gegenüber der Aquakultur positiv beeinflussen. Durch den Einsatz von spezies-spezifischen Fütterungs- und Handhabungszeiten könnte das Wohlergehen der Fische verbessert werden ohne dem Farmer zusätzliche Kosten aufzubürgen. Somit liefert meine Arbeit Ideen für neue Aquakultur Praktiken, die sowohl die Rentabilität der Zuchtbetriebe als auch die Wahrnehmung der Verbraucher verbessern können.

Chapter I

General Introduction

Introduction

Global Importance of Aquaculture

Aquaculture is defined as the rearing of aquatic organisms, including fish, molluscs, crustaceans, and aquatic plants and can be dated back to over 8000 years (Costa- Pierce, 2022; FAO, 2017; FAO, 2020; Levin, 2013; Rocha et al., 2022). It aims to increase production by intervening in the rearing process and can be broadly classified into three categories, namely intensive, semi-intensive, and extensive (Ackerfors et al., 2017; Campbell & Pauly, 2013; FAO, 2020). In this regard, intensive aquaculture is referred to as “modern aquaculture”, which underwent a technological evolution in the 1970s–1980s and utilises various techniques to regulate the growth processes, such as water quality management, supplemental feeding, and regulated stocking densities (Costa-Pierce, 2022; Føre et al., 2018). In contrast, extensive aquaculture uses only natural food sources and conditions, while semi-intensive aquaculture combines both to varying degrees (Ackerfors et al., 2017; Oddsson, 2020; Rocha et al., 2022).

In 2020, the global live weight produced in aquaculture (excluding aquatic plants) was about 87.5 million metric tons (mmt), of which 54.4 mmt were from inland aquaculture only, that is, land-based operations (Brugère et al., 2019; FAO, 2022; Mizuta et al., 2022). The contribution of aquatic species from global aquaculture production to seafood production reached an all-time high of 59.2% in 2020, while global landings of capture fisheries have stagnated since the 1980s, according to the Food and Agriculture Organization (FAO) (FAO, 2022; Garlock et al., 2019; Mizuta et al., 2022). This highlights the need to expand aquaculture production to keep up with the ever-increasing global demand for food and to meet animal protein needs (Brooks et al., 2019; Dawood et al., 2020; Garlock et al., 2019).

Global per capita consumption of aquatic animals increased by about 1.5% per year from 9.0 kg in 1961 to 20.5 kg in 2019 and is projected to increase to 21.4 kg in 2030 (FAO, 2022). In 2017, about 17% of the world population’s protein intake came from fish consumption (Ahmed et al., 2018; FAO, 2020). Taking a look at the distribution of aquaculture production in the world, Asia remains the world’s leading continent with

88.43% of total production, followed by America with 5% and Europe with 3.74% (FAO, 2022). Therefore, most seafood in the European Union (EU) is still imported or comes from wild fish stocks and should be replaced by sustainable, locally farmed fish to ensure food safety (de la Casa-Resino et al., 2021; Zander & Feucht, 2017). Landings of capture fisheries are stagnating, leading to a decline in the availability of (wild) fish to consumers and increasing the pressure on intensive fish farming and sustainable resources (de la Casa-Resino et al., 2021; FAO, 2020; FAO, 2022; Lyach & Čech, 2018).

Aquaculture, in general, can be applied in freshwater as well as in brackish or marine waters, the latter being the main form of production in European aquaculture (FAO, 2020; FEAP, 2020). In addition to marine aquaculture in nets and cages, there are other farming practices in European Aquaculture, situated in the open ocean, lakes, rivers, and ponds, as well as in land-based operations (Brooks et al., 2019; Buck & Langan, 2017; Buck et al., 2018; de la Casa-Resino et al., 2021; Mizuta et al., 2022; Rico et al., 2019; Wang et al., 2018). This diversification of aquaculture practices requires specific management skills related to the specific environment and economic as well as social resources (Bohnes et al., 2019; Cascarano et al., 2021; de la Casa-Resino et al., 2021; Fonseca et al., 2020; Oddsson, 2020). Hence, responsible consumption and sustainable fish production can only be achieved with sustainable management strategies tailored to each individual farming system (Froehlich et al., 2022; Henriksson et al., 2012).

The major challenges in today's intensive fish farming are farm sustainability, environmental challenges, animal welfare, as well as health issues, and consumer acceptance (Ahmed et al., 2019; Cascarano et al., 2021; Gentry et al., 2017). Advanced technologies and improved management strategies can support the eco-intensification of aquaculture and make them an economically competitive and sustainable food sector (Brooks et al., 2019; Garlock et al., 2019).

A promising and widely used solution for the future of intensive aquaculture is the use of recirculating aquaculture systems (RAS), which can increase productivity and reduce both environmental impacts and resource use (Ahmed et al., 2019; Badiola et al., 2018). Excessive harmful components in water, such as feed residues, faecal matter, ammonia, nitrite, bacteria, and carbon dioxide (CO_2), affect the aquatic product quality and must be removed (Ahmed & Turchini, 2021; Aich et al., 2020; Badiola et al., 2018). RAS are intensive land-based aquaculture farms that (partially) reuse water through various water treatment processes (Cristiano et al., 2022; Martins et al., 2010; Xiao et al., 2019). They use physical filtration (e.g. drum filter), biological filtration (e.g. biofilter with fluidised sand), water disinfection (e.g. ozone), and O_2 addition to ensuring a high-quality environment for fish (Bergheim & Fivelstad, 2014; Ruiz et al., 2020; Xiao et al., 2019). RAS are considered a good solution for sustainable eco-intensification because they minimise water consumption by recycling up to 90% of the water and allow controlled conditions for fish (Aich et al., 2020). Ensuring water quality and controlled parameters (like salinity, temperature, pH, or oxygen) results in optimised growth, directly reducing operating costs for feed (Ahmed & Turchini, 2021; Cristiano et al., 2022).

In general, eco-intensification is interpreted as a system that produces relatively higher yields on the same land area while reducing environmental impacts (Aubin et al., 2019; Cristiano et al., 2022; Godfray et al., 2010). Unfortunately, the need to improve aquaculture productivity has often led to an unhealthy intensification of the sector, resulting in poor animal welfare, resource misuse, and limited growth performance of farmed species (Dawood et al., 2018). To that end, the circular economy approach could be a solution to increase intensification while further reducing resources and improving fish welfare (Fraga et al., 2022). The circular economy is defined as "[…] an economic system that replaces the 'end-of-life' concept with reducing, alternatively reusing, recycling and recovering materials in production/distribution and consumption processes […], with the aim to accomplish sustainable development, thus simultaneously creating environmental quality, economic prosperity, and social equity, to the benefit of current and future generations" (Kirchherr et al., 2017). This combination of circular economy and RAS could allow more sustainable intensive production of fish (Campanati et al., 2022; Villar-Navarro et al., 2021).

Blue Growth

In order to tackle global challenges, such as hunger, malnutrition, and climate change, the Sustainable Development Goals (SDGs) have been introduced by the United Nations General Assembly in 2015 (Assembly, UG, 2015). These science-based management strategies have been commonly accepted as the minimum criteria for sustainable fisheries and aquaculture (Cavallo et al., 2021; FAO, 2020; Fonseca et al., 2020). The SDGs aim to ensure a better life for humanity by balancing economic, social, and environmental development (United Nations General Assembly, 2015; Fonseca et al., 2020). The United Nations (UN) has established a framework for “[…] peace and prosperity for people and the planet, now and in the future […]” (Assembly, UG, 2015). These SDGs have 17 main targets, of which six are particularly relevant to the aquaculture sector, namely Zero hunger (2), good health and well-being (3), decent work and economic growth (8), responsible consumption and production (12), climate action (13), and life below water (14) (Sachs et al., 2021). The fight against global hunger and the nutritious aspects of healthy living are directly linked to global animal production, terrestrial as well as aquatic.

Despite growing health and environmental concerns about processed meat, overall meat consumption has steadily increased since the 1960s. Therefore, a shift in mindset is needed to achieve the SDGs (González et al., 2020; Kwasny et al., 2022; Zeng et al., 2019). Intensive livestock production of terrestrial animals is a major driver of climate change, accounting for 14.5% of anthropogenic greenhouse gas emissions and contributing to deforestation, land degradation, as well as water and air pollution (Baabou et al., 2017; Cascarano et al., 2021; Froehlich et al., 2022; Godfray et al., 2018; Happer & Wellesley, 2019). Terrestrial animal agriculture consumes more freshwater than any other human activity and thus requires drastic reductions to meet the needs of the human population while protecting environmental resources (Assembly, UG, 2015; Aubin et al., 2019; Cascarano et al., 2021). Eliminating animal proteins and switching to a mostly plant-based diet is recognised to have the greatest influence to achieve the SDGs, but it is challenging due to ingrained cultural practices, societal norms, and daily habits (Kwasny et al., 2022). One potential solution may be meeting consumer demand for animal protein with more aquatic and less terrestrial animal protein sources to support the SDGs without abandoning animal-based diets (Folke et al., 1998; Fraga et al., 2022; Godfray et al., 2018; Guillen et al., 2019).

Responsible and sustainable aquaculture can therefore contribute to the achievement of the SDGs. Thus, the European Commission (EC) introduced the term “blue growth” in 2017, focusing on the pillars of sustainable development (FAO, 2017b). As part of its blue growth strategy, the EC identified the aquaculture sector as having the greatest potential for creating sustainable jobs, food security, and growth (European Commission, 2012). To make the aquaculture sector in Europe more sustainable, the introduction and promotion of CE and RAS are helpful.

This PhD thesis was conducted within the EU Horizon 2020 project GAIN (Green aquaculture intensification in Europe funding N° 773330), which started in 2018 and ended in 2021. The project firmly focused on investigating the potential of the eco- intensification of European aquaculture. Key aspects of the project were feed improvements, circular economy, policy, and markets (GAIN, 2020). In addition, research efforts focused on integrating scientific and technical innovations, new policy and economic instruments, and reducing social barriers, that is, identifying and addressing widespread negative perceptions of aquaculture products (Conceição et al., 2020; GAIN, 2020; Krause et al., 2020). As part of the GAIN project, this dissertation focuses on a transdisciplinary approach to improving European aquaculture practices by understanding consumer perspectives, improving novel feed ingredients, and analysing fish welfare.

Integration of social and natural sciences- Consumer perception of seafood products

The key to increasing the demand for aquaculture produced fish is a positive consumer attitude (FAO, 2022). Therefore, it is important to understand what influences this perception and how it can be shifted into a more positive direction. Generally, food perceptions are largely influenced by concerns about poor animal welfare, potential climate impacts, sustainability, health benefit, and farming conditions (Funk et al., 2021; Hoerterer et al., 2022a; Petereit et al., 2022a; Wongprawmas et al., 2022; Zander & Feucht, 2018). The general public continues to evaluate aquaculture seafood production negatively due to serious ecological and environmental problems in recent decades (Altiok et al., 2021; Bogard et al., 2019; Ruiz et al., 2019; Wongprawmas et al., 2022). The greatest concerns involve the proportion of wild-caught fish in aquaculture feeds and the welfare of farmed animals (Bogard et al., 2019; Farmery et al., 2022).

Consequently, alternative feeds and improving fish welfare are the most important factors in transforming consumer perceptions towards more favourable attitudes. This dissertation analyses and discusses both of these aspects in greater depth. Consumer perspectives on seafood are examined first in more detail to better understand and contextualise the underlying background.

In the context of a healthy and sustainable diet, seafood consumption has the potential to play an essential role in human nutrition due to its health-promoting properties and high-protein content (Balami et al., 2019; FAO, 2022; Pal et al., 2018; Pieniak et al., 2008). Among other things, fish products may lower the risk of heart attacks, protect against certain types of cancer and thrombosis, decrease blood clotting tendencies, and enhance child growth and development (Bogard et al., 2019; Geelen et al., 2007; Mori et al., 1997; Pieniak et al., 2008). Moreover, fish provide a broad range of nutritional benefits, including vitamins, minerals, fatty acids, and high-quality proteins (Bogard et al., 2019; De Boer et al., 2020; Pal et al., 2018).

Intensive fish farming has the greatest transformative potential for sustainable consumption and food security in Europe (Eurobarometer, 2019; FAO, 2020; Hackenesch et al., 2016). However, consumers distrust fish from intensive aquaculture because of environmental risks, unrestricted use of antibiotics, and unsustainable use of resources, such as the exploitation of wild fish stocks (Godfray et al., 2018; Mog et al., 2020; Naylor et al., 2020; Pieniak et al., 2008). It is believed that the media's false, outdated, and misleading information often shape negative consumer attitudes in Europe (Govaerts, 2021; Hoque et al., 2020; Pulcini et al., 2020). Antibiotics, for instance, have been subject to strict regulations since 2006 based on drug resistance, food safety, and environmental risks and are now used only when necessary (Assefa & Abunna, 2018; Burridge et al., 2010; Lulijwa et al., 2020; Sapkota et al., 2008). These misconceptions might cause knowledge gaps among consumers and thus are a potential contributing factor to the low consumption rates of farmed fish in Europe compared to Asia (Banovic et al., 2019; Boase et al., 2019; Gaviglio A., 2009; Menozzi et al., 2020). Consumer education on RAS could help improve public perception as less antibiotics need to be used in controlled farming systems, resulting in less environmental impact and better fish health attributes (Badiola et al., 2018; Cristiano et al., 2022).

Unfortunately, consumers' perceptions do not always match their claims, making interpreting of purchasing behaviour a complex subject. The literature highlights that the major drivers of seafood purchases are demographics, knowledge, net income, and personal background, such as traditions and culture (Baabou et al., 2017; Boase et al., 2019; Funk et al., 2021; Jonell et al., 2016; Petereit et al., 2022a; Verbeke et al., 2007; Vinayak and Arora, 2018; Yi, 2019). Consequently, these factors need to be considered when promoting sustainable ecological intensification of aquaculture production (Raworth, 2017; Wiedmann & Lenzen, 2018). However, neither demographics, net income, nor traditions can be easily changed through campaigns or policies. Therefore, the only thing that can be proactively changed among consumers is to increase awareness and knowledge about seafood. Accordingly, it is essential to determine the level of knowledge at the individual consumer level and how perceptions can be positively and more sustainably influenced through future campaigns (Boase et al., 2019; De Boer et al., 2020; Funk et al., 2021; Hoerterer et al., 2022a; Petereit et al., 2022a; Pulcini et al., 2020; Wongprawmas et al., 2022).

An emerging aspect of sustainable consumption is "green purchasing" (Lucas et al., 2018; Yi, 2019). Consumers are increasingly developing environmentally friendly behaviours that lead to the purchase of so-called green products. These products are sustainable, regionally produced, and environmentally friendly (Brécard et al., 2009; Jonell et al., 2016; Yi S., 2019). In contrast to these green purchasing attitudes is the volume of actual purchased products, indicating that price remains the main barrier to sustainable consumption (Asche et al., 2021; Lucas et al., 2018). Several studies have shown that consumers have a maximum price premium. They are willing to pay extra for seafood products labelled as sustainable (Ankamah-Yeboah et al., 2016; Asche & Bronnmann, 2017; Bronnmann & Asche, 2016). This is in the range of around 20%, meaning anything above this level is rejected by consumers (Zander & Feucht, 2017). Unfortunately, majority of studies focus on overall seafood products and do not differentiate between fishery and aquaculture products, creating a data gap (Asche et al., 2021; Farmry et al., 2022; Jonell et al., 2016).

In addition to price, feeding methods and product's origin appear to be major factors for consumers (Alfnes et al., 2018; Jonell et al., 2016; Lawley et al., 2019). Szendro et al. (2020) have shown research indicating dual importance of feed selection and animal origin, inclusive of rearing method (wild-caught, RAS, ponds, and so on), to consumers. Consequently, aquaculture farmers should focus both on the rearing system and the type of feed used for the fish. Furthermore, consumers are often still subjected to the misconception that wild-caught fish is healthier than fish from aquaculture (Banovic et al., 2019; Schlag & Ystgaard, 2013; Thong & Solgaard, 2017). On the contrary, fish from land-based aquaculture generally contains fewer toxic elements in the final product than wild fish, as hazardous substances from the sea bioaccumulate in wild fish (Ahmed & Turchini, 2021; Cahu et al., 2004; Hamada et al., 2018; Schlag & Ystgaard, 2013). This is yet another reason why farmed fish from RAS may be a promising option for safe seafood consumption in the future.

Consequently, educational campaigns that reflect how animals are raised are an excellent tool to raise awareness about animal welfare and their origins, including their farming conditions (Potter et al., 2021). One such management measure is the eco- label, a market-based economic tool designed to guide consumer purchasing behaviour towards aquaculture (Alfnes et al., 2018; Madin & Mukaratirwa, 2015). The criteria of a label can address environmental, social, and economic objectives (Cantillo et al., 2020). Therefore, eco-labels have become an essential strategy of environmental organisations and have been proven to impact consumers' behaviour (Madin & Mukaratirwa, 2015; Potter et al., 2021). They encourage consumers to consider sustainable products more and further motivate direct purchase of these labelled products (Zander & Feucht, 2018). More than 230 eco-labels are listed and used in European trade, including 48 for aquaculture products alone (Ecolabel index, 2022; Asche et al., 2021). This wide variety of eco-labels is counterproductive and can be irritating to consumers (Asche et al., 2021; Petereit et al., 2022a; Potter et al., 2021). The most prominent eco-label in Europe is a private certification scheme based on FAO guidelines developed in 2010, the Aquaculture Stewardship Council (ASC) (FAO, 2022). It was the first eco-label used to promote sustainable aquaculture and consumption campaigns (Yi S., 2019). Common to most labels is that they use life cycle assessment (LCA) to determine the sustainability and impact of a product (Alfnes et al., 2018; Cordella et al., 2020; Mungkung et al., 2006). LCA evaluates the domestic and international impacts of a product, that is, material consumption, infrastructure, energy, and all steps associated with the product manufacturing process (Cao et al., 2013; ISO 2006, Martins et al., 2010; Wilfart et al., 2013). Usually, LCA is divided into four steps: a) definition of the system and its boundaries; b) translation of data into environmental impact indicators; c) results and analysis; and d) interpretation (Bergman et al., 2020; Cao et al., 2013; Martins et al., 2010). LCA highlights that sustainable intensive fish farming needs to increase feed conversion ratio (FCR) (using alternative feeds), minimise external inputs (e.g. using RAS), and increase the use of renewable resources (e.g. circular economy) (Mungkung et al., 2006; Wilfart et al., 2013). Through the use of life cycle assessments conducted under eco-labels, consumers can self-assess the sustainability of a product and adjust their purchasing behaviour based on that information. LCA can include several categories useful to consumers, such as global warming potential, energy use, marine aquatic ecotoxicity, carbon footprint, water use, biomass reuse, or acidification (Bergman et al., 2020; Bohnes et al., 2019; Cordella et al., 2020; Cristiano et al., 2022; Henriksson et al., 2012).

According to the LCA, feed has the greatest impact on all of the mentioned indicators (Bergman et al., 2020; Cao et al., 2013; Cordella et al., 2020; FAO, 2022; Glencross, B. D., 2020; Mungkung et al., 2006). An explanation for this is that most carnivorous fish feeds consist of fishmeal and fishoil, which are often perceived as unsustainable resources (FAO, 2020; Martins et al., 2010). This initiates the second part of my research, which addresses alternative feed concepts for improving sustainability and enhancing the performance parameters while guaranteeing the nutritional status and welfare of the fish in RAS.

Sustainable fish feed ingredients

In addition to positively influencing consumer perceptions, the expansion of intensive fish farming also requires innovation and improvement of commercial fish feed. Feeding fish in intensive aquaculture systems impacts growth rates, animal welfare, as well as animal health and accounts for up to 70% of total production costs (Dawood, 2020; Henry et al., 2015). Therefore, multiple parameters must be considered when formulating alternative feed designs to maximise yield, increase environmental friendliness, and remain affordable to the farmer (Bennett et al., 2021; Hoerterer et al., 2022b; Petereit et al., 2022b).

In general, fish feed depends on the provision of essential nutrients, such as high-quality proteins, to promote growth and animal health (Bandara, 2018). The major protein sources in commercial diets for carnivorous fish are fishmeal and fishoil (FAO, 2020), primarily because of their high-protein content of up to 73% and their balanced essential amino acid (EAA) profile, which is necessary for fish health and growth (Bandara T., 2018; FAO, 2022; Luthada-Raswiswi et al., 2021). Fishmeal and fishoil are often extracted from marine fish stocks and have many drawbacks, such as high prices, supply shortages, or quality problems (Henry et al., 2015; Mahamud et al., 2022). It is worth noting in this context that fishmeal from marine fish is still perceived by consumers as unsustainable, but for many parts of the world this is not the case. Especially Peruvian anchoveta (*Engraulis ringens*) fisheries have come to be considered sustainable through various management strategies (Canales et al., 2021; Pariona-Velarde et al., 2020). Nevertheless, fishmeal from marine fish stocks often faces quality problems from increased environmental impacts, such as microplastics and heavy metals, as well as high carbon footprint from long transport distances (Dağtekin et al., 2022; Loaiza et al., 2022; Pariona-Velarde et al., 2020). This is especially important in grave situations, such as global pandemic (e.g. COVID-19 or other diseases), political escalations (e.g. wars or other forms of conflict), or natural phenomena and disasters (El Nino, tsunamis, and others), which increase the need for autonomy (FAO, 2022; Mangano et al., 2022; Mirto et al., 2022). In the last years, aquaculture production faced challenges as supply chains collapsed, resulting in transportation bottlenecks (Ahmed & Azra, 2022; Carbonara et al., 2021; FAO, 2022). Therefore, sustainable, locally sourced, and cost-effective ingredients are required to meet the increasing demand for feed and reduce dependency on unsustainable resources (FAO, 2022).

Nutrient requirements vary among fish species, affecting both growth and stress parameters and consequently need to be addressed at a species-specific level (Luthada-Raswiswi et al., 2021). In this study, adult European seabass (*Dicentrarchus labrax*) was used as a model fish for feeding experiments in RAS to determine the effects of alternative feed ingredients. Seabass is the most important aquaculture species in the Mediterranean Sea, ranking second after salmon in highest per capita consumption

(FAO, 2022; Llorente et al., 2020). Together with gilthead seabream (*Sparus aurata*), seabass accounts for over 90% of fish production in the Mediterranean and has a long history of aquaculture, making it a very well-researched species (FEAP 2017; Rigos et al., 2020). Since it is a carnivorous fish, the protein requirement is high and must be considered while formulating new diets (Petereit et al., 2022b). By-products, plant compounds, and insects appear to be promising solutions to increase the sustainability of fish diets while ensuring growth and health (Bandara, 2018; Hoerterer et al., 2022b).

The most promising solutions for fishmeal substitution are aquaculture and fishery by-products, along with hydrolysates (Al Khawli et al., 2019; Hua et al., 2019; Siddik et al., 2021). By-products are defined as substances that are obtained secondary as part of an integrated primary production process and can be reused directly without any additional processing steps, provided that they meet all regulatory requirements (Fraga et al., 2022). Some examples are damaged fish, wastes, as well as other body parts unsuitable for human consumption (Campanati et al., 2022; Siddik et al., 2021). Aquaculture, along with fishery product wastes, has significant potential to serve as an alternative protein source. However, these wastes, which represent up to 60% of production, are usually converted into low-value products, such as fishmeal or fertilizers (Siddik et al., 2021). A more viable option to process these waste materials would be so-called fish protein hydrolysates (FPH). FPH are processed raw materials that are more energy efficient than byproduct fishmeal (Hoerterer et al., 2022b; Siddik et al., 2021). Fish protein hydrolysates are degradation products of the enzymatic hydrolysis of proteins into smaller peptides (Desai et al., 2022; Pariona-Velarde et al., 2020). This process reduces the peptide size to approximately 2 to 20 amino acids, making hydrolysates a source with excellent physicochemical properties, such as increased solubility and fat-binding capacity, and resulting in improved feed digestion and nutrient uptake (Gajanan et al., 2016; Malcorps et al., 2020; Pariona-Velarde et al., 2020; Siddik et al., 2021).

Besides aquatic animal by-products, several terrestrial animal by-products are also evaluated, including potential feed ingredients for aquatic diets, such as poultry meal, blood meal, and feather meal (Bandara, 2018; Luthada-Raswiswi et al., 2021). Their advantages are fewer wasted resources, cheaper rate, prevalence of nutrient-friendly compounds, and ease of production (Forster et al., 2006; Napier et al., 2020). One negative aspect of these by-products are the lack of nutrients and EAAs for the fish, as well as strict European regulations on their use (Bandara, 2018; Luthada-Raswiswi et al., 2021). These stringent guidelines resulted from severe animal diseases recorded in the past, such as bovine spongiforme Enzephalopathie (BSE) and avian influenza (Luthada-Raswiswi et al., 2021).

Plant proteins are another attractive alternative to animal by-products because of their high lipid and protein content (Beal et al., 2018; Luthada-Raswiswi et al., 2021). However, plant-derived proteins have an unbalanced amino acid (AA) profile and nutrient deficiencies for carnivorous fish, which can potentially cause inflammation in the digestive tract (Beal et al., 2018; Bandara, 2018; Henry et al., 2015; Jannathulla et al., 2019). This can be ameliorated, though, by either combining different types of plants or by adding essential AAs to compensate for the deficiency (Beal et al., 2018; Bandaga, 2018). A further issue with crops is their sustainability and ecological footprint (Banerjee et al., 2021). Since human population is growing, more and more arable land is being utilised, with agriculture competing for land use, water availability, and energy, making some crops decreasingly sustainable for fish feed (FAO, 2022; Henry et al., 2015).

The main vegetable ingredient in most commercial fish feeds is soya bean, which is often utilised as soybeanmeal (SBM). In recent decades, SBM has become an important alternative protein source in aquaculture, owing to its high protein content of up to 50%, faster growth rates, and low prices (Henry et al., 2015; Pereira et al., 2020). One the other hand, antinutritional factors in soya bean, such as sapions and phytic acid, reduce the production of important enzymes in fish and negatively affect the digestion of proteins and lipids (Bandara, 2018; Beal et al., 2018). These can be counteracted by the inclusion of EAAs and other additives, as has been done with other plant species (Dawood etal., 2018; Hua et al., 2019; Jannathulla et al., 2019). However, in recent years, sustainability concerns have surfaced as the extensive and ever-growing cultivation of soy has led to deforestation and loss of biodiversity, as well as other major environmental and social problems (Carbonara et al., 2020; Pereira et al., 2020). In fact, 60% of the global deforestation is associated with SBM and derivatives consumed by EU countries (European Technical Report, 2013). This dependency of European countries makes the market highly vulnerable to price volatility and trade distortions, especially in terms of global crises (Ahmed & Azra, 2022; Mangano et al., 2022; Pereira et al., 2020).

A more independent alternative to terrestrial plants are microalgae, which can easily be produced at low costs (Oostlander et al., 2020). Microalgae represent a natural food source of marine species and hold great potential for replacing fishmeal and fishoil with up to 60% of protein content, as well as multiple other valuable nutrients (Beal et al., 2018; Nagarajan et al., 2021). Furthermore, they can support the maintenance of water quality in RAS, and their antioxidants may improve stress tolerance in animals (Han et al., 2019; Nagarajan et al., 2021). However, microalgae are a relatively new component in aquaculture, and continued research is needed to evaluate their full potential.

Another relatively novel ingredient for fish feeds is insects, a natural diet for both freshwater and marine species, which leave a small ecological footprint and require only a little space (Henry et al., 2015; Luthada-Raswiswi et al., 2021; Szendrő et al., 2020). In addition, insects are high in amino acids, lipids, minerals, vitamins, and proteins (50– 82% of dry matter), which makes them a promising alternative for fishmeal and fishoil (Bandara, 2018; Henry et al., 2015). Since they grow on terrestrial agricultural waste, insects require nearly no land or water and generate low carbon emissions while biodegrading as waste products (Bandara, 2018). The main problem, however, is consumer perception and laws in Europe, which still hamper the commercially viable cultivation of insects (Lawley et al., 2019). Raising awareness of alternative ingredients is thus an important tool to support sustainable fish aquaculture and increase its consumption.

Fish welfare in RAS

The implementation of enhanced animal welfare has become a top priority for farmers due to increasing pressure on well-functioning and intensive fish farming (Ceinos et al., 2019; Martos-Sitcha et al., 2020; Ytrestøyl et al., 2020). However, intensive livestock production causes stress and affects the health, survival, and growth of animals, resulting in overall poor welfare (Ashley, 2007; Toni et al., 2019). This low welfare, in turn, affects farm productivity and negatively impacts consumer perceptions, which again leads to decreased sales.

The understanding of fish welfare and consideration of this aspect in aquaculture farms is relatively new. In 1992, the UK Council for Farm Animal Welfare established five freedoms for the protection of animals on farms, which were expanded in 2006 by the European Convention for farmed fish (Council Farm Animal Welfare, 1992; Council of Europe, 2006). Moreover, in 2010, Directive 2010/63/EU established guidelines for the protection of animals used for scientific purposes, stating that "[…] vertebrate animals including cyclostomes, cephalopods, need to be protected […] as there is scientific evidence of their ability to experience pain, suffering, distress and lasting harm " (Stien et al., 2020). This guideline ensures that all animals used for human purposes are provided with food, water, appropriate environment, and care according to their species-specific health and well-being.

To ensure fish welfare, a precise biological knowledge of the species is mandated (Toni et al., 2019). Assessing welfare requires multi-level approaches due to species dependency, high individual variability, and interwoven complex metabolic processes (Ashley, 2007; Carbonara et al., 2020; Martos-Sitcha et al., 2020; Tort, 2011). Indicators of welfare are, for instance, health status, behaviour, water quality, and specific metabolic compounds, including cortisol, glucose, and lactate (Barreto et al., 2022; Martos-Sitcha et al., 2020; Vis et al., 2020). Such factors make it imperative to study each species individually to obtain the best possible results for the farmer. The last chapter of this dissertation explicates a circadian rhythm (CR) experiment that determines the first trend in the day and night rhythm of turbot (*Scophthalmus maximus*) and evaluates its best aquaculture practices.

Turbot is a valuable marine fish with a production volume of 8.395 mmt in the EU are high prices and is appreciated by consumers for its white, mild-tasting filet, usually low in fat (2–4%) (FAO, 2020; Fraga-Corral et al., 2022; Malcorps et al., 2020; Pyanov et al., 2021). Turbot are typically cultured in RAS and the growth and health of this species have been well studied (Fraga-Corral et al., 2022; Hoerterer et al., 2022b). However, the stress physiology within this species remains poorly understood and in addition, to date, no study has addressed the CR of turbot. Extrapolating from one species to another is highly speculative as stress physiology varies strongly not only in intraspecific but also interspecific cases (Ashley, 2007; Barton, 2002; Ellis et al., 2011; Pankhurst, 2011).

Therefore, it is necessary to determine the circadian rhythm of each aquaculture species to achieve the best species-specific performances.

Increasing concern for animal welfare has resulted in the expansion of research to define and sustainably minimise stressful situations for fish. In this context, the circadian rhythm in aquaculture has become an emerging issue. The biological system underlying the CR controls every living organism and greatly influences their metabolism, and consequently, growth, feeding, and health (Prokkola et al., 2018; Sanchez-Vazquez et al., 2019). Therefore, understanding the circadian rhythm of aquaculture fish is critical to maximize productivity and optimizing yield and welfare. The CR consists of multiple molecular processes as well as several clock genes such as *per, clock, bmal*, and others (Pilorz et al., 2018; Sanchez- Vazquez et al., 2019). These clock genes synchronize all metabolic functions according to the environmental conditions to increase the fitness of the animal (Prokkola et al., 2018). The CR is thus responsible for numerous cellular processes, including stress and immune responses as well as appetite (Ceinos et al., 2019; Nikkhah, A., 2015; Pilorz et al., 2018; Prokkola et al., 2018: Zheng et al., 2021). Previous studies have shown that reduced feed intake in fish is a distinct behavioural response to stress and affects not only growth but also their welfare and body composition, and thus yield and profitability for the farm (Assan et al., 2021; Dawood et al., 2020; Lopez-Olmeda et al., 2009; Yang et al., 2018). Choosing the right feeding time according to the animal's natural metabolism is therefore important for both physiological and economic reasons. The CR is driven by biotic and abiotic parameters and is particularly sensitive to changes in light (Choi et al., 2020; Pilorz et al., 2018; Sanchez- Vazquez et al., 2019). Hence, understanding CR is critical for intensive aquaculture farms, which manipulate abiotic factors, such as light, temperature, salinity, etc., to increase efficiency, especially in RAS (Choi et al., 2020; Prokkola et al., 2018).

Of special interest to intensive fish farming is that food intake and utilization can vary significantly even within a 24-hour cycle and are regulated by endocrine mechanisms and are strongly influenced by stress (Assan et al., 2021; Barton, 2002). Stress is generally defined as the disruption of physiological or biological mechanisms caused by internal and external factors commonly referred to as stressors (Barton, 2002; Ramsay et al., 2009; Wendelaar Bonga, 1997). In intensive fish farms, fish are exposed to many different stressors, such as high stocking densities, transportation, or restricted and unfamiliar environments (Long et al., 2019; Martos-Sitcha et al., 2020). Previous studies have shown that reduced feed intake in fish is a distinct behavioural response to stress and affects growth and fish welfare (Assan et al., 2021). However, in most commercial fish farms, animals are fed according to fixed feeding schedules instead of adapting to the animals' metabolism in a species-specific manner (Callier et al., 2017; Pratiwy et al., 2021; Pilorz et al., 2018). Therefore, an optimized feeding regime could improve feed conversion at no additional cost to the farmer, which results in higher profitability. Moreover, avoiding feed wastage through proper feeding minimizes the loss of expensive feed and the accumulation of nitrogenous wastes in the water, which could be toxic to fish (Assan et al., 2021).

There are many indicators to analyze stress in fish, and cortisol is among the most established and commonly used in circadian rhythm studies (Cowan et al., 2017; Prokkola et al., 2018; Tort, 2011; Valencuela et al., 2022). However, in addition to cortisol as a stress marker, glucose is critically vital in aquaculture operations due to its influence on animal nutrient availability and oxygen consumption (Prokkola et al., 2018). Oxygen availability is often a limiting factor in intensive farms, as well as an optimized feed conversion ratio to improve productivity (Assan et al., 2021). Furthermore, together with other sugars, glucose is the main energy supplier of the organisms since it is metabolized into adenosine triphosphate (ATP) during gluconeogenesis (Cowan et al., 2017). That is important as the availability of energy in the organism helps the animal to better cope with stressful situations (Valenzuela et al., 2022).

Furthermore, previous studies have demonstrated a close link between fish's immunological and stress responses and, consequently, circadian rhythms (Montero et al., 2019; Schleiermann et al., 2013; Valenzuela et al., 2022). Stress suppresses immune responses when fish are exposed to stressors such as feed intake, handling, or care/transport practices, and as a result, increases the risk of disease outbreaks (Hernández-Pérez et al., 2019; Sakai et al., 2021; Skouras et al., 2003; Song et al., 2021). Immune and stress parameters are related to the natural metabolism of fish and change within seasonal and daily fluctuations (Subbotkin & Subbotkina, 2021). Therefore, it is important to look at both stress and immune parameters, and one well- studied immune component in fish are lysozymes (Smith et al., 2019; Song et al., 2021; Subbotkin & Subbotkina, 2021). Lysozymes play an essential role in the innate immune system, and blood concentrations are directly linked to concentrations in the liver (the major lysozyme-producing organ) (Valenzuela et al., 2022). These are enzymes with antimicrobial activity, i.e., they eliminate pathogens in the organism and show decreased levels during times of high stress (Shakoori et al., 2018; Song et al., 2021). Lysozymes are part of the non-specific immune lineage and, like all immune responses, have a high energy expenditure (Subbotkin & Subbotkina, 2021). This has particular implications for fish health, and a better understanding could reduce the risk of disease outbreaks and lower stress levels as well as increase food intake and utilisation in aquaculture operations. Therefore, it is crucial to know the processes that occur over a 24-hour period in order to adjust the feeding timing (during stressed and non-stressed periods throughout the day) to the metabolism of the fish. Consequently, knowledge of these processes could contribute to improve farm productivity by optimising the feeding time of the animals, reducing their stress, and consequently increasing welfare and farm profitability.

To understand these molecular metabolic processes of an organism, metabolomics is a powerful analysis to identify its key pathways (Deborde et al., 2021; Hatzakis, 2019). Metabolomics is the study of endogenous metabolite profiles in biological samples, like body fluids such as plasma and serum (Macias et al., 2019; Samuelson et al., 2006). It is and an effective approach to elucidate the interactions between aquatic organisms and their environment (Samuelson et al., 2006), discover biomarker profiles (Macias et al., 2019), and gain deep insights into mechanisms associated with circadian rhythms in fish (Figueiredo et al. 2020; Lopes et al., 2022; Montero et al., 2019). Similar to any living organism, fish are composed of distinct molecules, such as lipids, water, proteins, carbohydrates, vitamins, amino acids, and other metabolomic compounds that can be measured using nuclear magnetic resonance (NMR) spectroscopy (Hatzakis, 2019; Steinsholm et al., 2020). The application of this technique is relatively new in the biological field and has rapidly increased over the past 20 years (Capello, 2020; Crook & Powers, 2020; Tavares et al., 2022).

NMR spectroscopy is a qualitative analytical tool commonly used for metabolomics and becoming increasingly important for aquatic species (Crook & Powers, 2020; Deborde et al., 2021; Roques et al., 2021; Tavares et al., 2022). Considered a non-destructive method of analysis, it allows for high reproducibility, qualitative metabolic profiling, rapid analyses, and cost efficiency (Capello, 2020; Hatzakis, 2019). Among the other benefits of NMR are its unbiased assessment for metabolite data, making it an ideal tool for non-targeted analyses (Tavares et al., 2022; Young & Alfaro, 2016). NMR spectroscopy provides information on the overall metabolic state of an animal and can be implemented in cells, tissues, organs, plasma, or even whole organisms (Capello, 2020; Crook & Powers, 2020; Roques et al., 2018). The majority of NMR-based environmental metabolomics studies focus on ^{1}H NMR spectroscopy to investigate the metabolome of a broad range of samples, both biofluids and tissues (Aru et al., 2021). In broad terms, NMR spectroscopy utilises signal intensity to determine the number of individual nuclei within a sample (Cullen et al., 2013). This enables the identification of a quantifiable measure of molecules and hence underlying metabolic processes, such as the CR (Deborde et al., 2021; Roques et al., 2021).

Thesis objectives

This dissertation focuses on the relationship between consumer perceptions and the development of alternative feed concepts, as well as fish welfare in recirculating aquaculture systems. **Chapter II** provides an overview of the publications produced during my PhD, and the following three chapters present my first authored manuscripts. **Chapter VI** provides a discussion of my findings, implications for aquaculture, limitations of my work, and final recommendations for the future.

I seek to improve the overall understanding of sustainable aquaculture in Europe through the following objectives, which are addressed in **Chapters III, IV and V**, respectively.

Objective 1 (Chapter III): My first objective was to investigate consumer perceptions of seafood at various knowledge transfer events. I aimed to determine both country- specific differences as well as the state of scientific knowledge in order to address the following research questions: Does more scientific knowledge of seafood products increase consumer awareness? Does having more scientific knowledge bear the potential to positively change purchasing behaviour towards sustainable aquaculture products? How do country-specific differences affect seafood perception? More precisely, how can we positively influence consumer perceptions and acceptability of more sustainable aquaculture products?

Objective 2 (Chapter IV): Alternative feed concepts are needed to ensure sustainable production in intensive fish farms and life cycle assessment, described feed as the most critical influencing factor of all parameters relevant to consumers. Therefore, I evaluated the effects of alternative feed concepts for European seabass (*Dicentrarchus labrax*) in RAS and focused on the following research questions: Do alternative, more sustainable feed concepts provide the same parameter output for seabass relative to the commercial formulation of feed? Can sustainable ingredients derived through circular economy be the solution for improving productivity while being more environmentally friendly?

Objective 3 (Chapter V): In addition to sustainable production, consumer perception also focuses on fish welfare, which is affected through metabolism. An internal clock is responsible for seasonal and daily variations of the metabolism, including digestion and growth. This internal clock system, called the circadian rhythm, fluctuates over a 24-hour period, is dependant on abiotic factors and is highly species-dependent. In turbot, the diurnal and nocturnal rhythms have never been studied from a metabolomic perspective. Therefore, in the final part of my dissertation, I will focus on the blood analysis of key metabolic elements in turbot held in RAS. My aim is to gain a baseline understanding into its circadian rhythm and address the following research questions: How does the circadian rhythm of turbot vary over a 24-hour period? What are the potential implications for aquaculture practices in the future?

Chapter II

This dissertation is the culmination of four years of ongoing research and is highlighted by three first-author manuscripts in various stages of publication

Publication I –III

1st author Papers, included in this thesis

Publication I

Jessica Petereit , Christina Hoerterer, Gesche Krause (2022)

Country-specific food culture and scientific knowledge transfer events–Do they influence the purchasing behaviour of seafood products?

Aquaculture, 738590. https://doi.org/10.1016/j.aquaculture.2022.738590

The design of the experiments was developed by the candidate and the other co- authors. The candidate run the experiment together with the second author. The candidate performed the lab work and statistical analysis of the data. The candidate, together with the second and last author, wrote the manuscript which was revised by all the co-authors.

Publication II

Jessica Petereit , Christina Hoerterer, Adrian A. Bischoff-Lang , Luís E. C. Conceição ,Gabriella Pereira, Johan Johansen , Roberto Pastres and Bela H. Buck (2022)

Adult European Seabass (*Dicentrarchus labrax*) Perform Well on Alternative Circular-Economy-Driven Feed Formulations

Sustainability 2022, 14,7279. https://doi.org/10.3390/su14127279

The design of the experiments was developed by the candidate and the other co- authors. The candidate run the experiment together with the second author. The candidate performed the lab work and statistical analysis of the data. The candidate, together with the second and last author, wrote the manuscript which was revised by all the co-authors.

Publication III

Jessica Petereit , Björn Baßmann, Christian Bock, Gisela Lannig, Bela H. Buck (2022)
Circadian rhythm in turbot (*Scophthalmus maximus*): Daily variation of blood metabolites in recirculating aquaculture systems
Manuscript in preparation; Journal to be decided.
The design of the experiments was developed by the candidate and the other co- authors. The candidate run the experiment alone. The candidate performed the lab work and statistical analysis of the data with the second, third and fourth author. The candidate wrote the manuscript which was revised by two co-authors so far.

Publication IV – VII

Collaborated Papers, partly included in the annex

Publication V (included in annex 4)

Christina Hoerterer, Jessica Petereit, Gesche Krause (2022)
Informed choice: The role of knowledge in the willingness to consume aquaculture products of different groups in Germany.

Aquaculture, 556, 738319.

Publication IV (included in annex 5)

Christina Hoerterer, Jessica Petereit, Gisela Lannig, Johan Johansen, Gabriella V. Pereira, Luis E. C. Conceição, Roberto Pastres, Bela H. Buck (2022)
Sustainable fish feeds: potential of emerging protein sources in diets for juvenile turbot (*Scophthalmus maximus*) in RAS
Aquaculture International, 1-24. https://doi.org/10.1007/s10499-022-00859-x
The candidate designed the experiment with the first and last author. The candidate performed the feeding and sampling part of the experiment, the data analysis of that part and the lab work for several parameter. The candidate wrote the manuscript in close cooperation with the first author. All the authors revised the final manuscript.

The candidate designed the experiment with the first and last author. The candidate performed the data gathering, the data analysis of that part and wrote the manuscript in close cooperation with the first author. All the authors revised the final manuscript.

Publication VI (Manuscript version not included)

Christina Hoerterer, Jessica Petereit, Gisela Lannig, Johan Johansen, Luis E. C. Conceição, Bela H. Buck (2022)

Effect of diets of plant and animal protein sources and replacement level on growth and feed performance and nutritional status of market size turbot (*Scophthalmus maximus*) in RAS

Under Review in Frontiers in Marine Science - Marine Fisheries, Aquaculture and Living Resources, special issue: Integration of Sustainability, Preservation of Biodiversity and Conservation Goals in Aquaculture

The candidate designed the experiment with the first and last author. The candidate performed the data gathering and the data analysis with the first author. The candidate and all the other co-authors revised the final manuscript.

Publication VII (not included)

Krause, G.; Hoerterer, C., Petereit, J. (2020)

Report on consumer and stakeholder acceptance of eco-intensification measures, including impact assessment of improved information availability. Deliverable 3.7. GAIN - Green Aquaculture Intensification in Europe. EU Horizon 2020 project grant nº. 773330. 74 pp.

The candidate conducted data collection, data analysis, an intensive literature research, and report design with the first and second author. The candidate and all other co- authors revised the final manuscript.

Chapter III

Country-specific food culture and scientific knowledge transfer events – Do they influence the purchasing behaviour of seafood products?

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Country-specific food culture and scientific knowledge transfer events – Do they influence the purchasing behaviour of seafood products?

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ABSTRACT

A positive perception of aquaculture products is essential to boost production by using more sustainable and eco- friendly solutions. However, consumer perception and resulting purchasing decisions remain poorly understood. In most European countries, the consumer perception tends to be rather negative, which is reinforced by knowledge gaps and misleading information from the media. This is believed to have the greatest impact on the current low consumption rate of farmed fish across Europe. Previous research has suggested that consumers may often be reluctant to change their seafood purchasing behaviour despite having a solid scientific understanding of aquaculture products and their mode of production. In this study, we investigated the extent to which country- specific contexts and degree of scientific knowledge contribute to the purchasing behaviour of consumers across Europe. To this end, interactive poster surveys and semi- structured interviews were conducted at eight different knowledge transfer events (KTEs) across three countries, targeting 383 participants. The application of a yet underutilized method, an interactive poster survey, underscored the need to use new approaches to tackle consumer behaviour.

Our results indicate that increased scientific knowledge does not lead to changes in purchasing behaviour per se. Perceptions and purchasing habits are very contextual and vary from culture to culture. This points to the highly interlinked nature of country-specific marine food culture that ranges between individual awareness, scientific knowledge, and socio-cultural contexts, all of which renders in resulting individual purchasing decisions. Our results suggest focusing more on the sustainability of a product and emphasising the ongoing transition towards a circular economy approach in the aquaculture sector may be a promising pathway to foster more sustainability-driven purchasing decisions in the seafood sector. Our findings also question whether trying to educate the public about more sustainable purchasing criteria is really the key to foster more sustainable consumption patterns or whether we are working from misleading assumptions that lead to wrong approaches. In conclusion, a lack of clear and easily accessible information appears to be the main barrier to social acceptance of sustainable aquaculture products in Europe.

Introduction

Roughly 60% of the world's marine fish stocks are fully exploited, and 33% are overfished all of which call for transformation in the sea- food sector (FAO, 2018, López-Mas et al., 2021). This is especially timely in the European Union, where consumers use three times more seafood than is produced, making Europe the largest importer of seafood in the world (Kresic et al., 2020).

Under this umbrella, marine aquaculture is believed to boost the highest transformative potential towards more sustainable pathways of future food security (Altintzoglou et al., 2020; Eurobarometer, 2018; Krešić et al., 2020; Hackenesch et al., 2016).

One approach to promote sustainable aquaculture growth is the concept of eco-intensification, i.e., production sufficient to meet the needs of the human population while respecting environmental needs and promoting health benefits (Aubin et al., 2019; O'Donncha and Grant, 2019; Pieniak et al., 2010). Eco-intensification is a challenge that requires the integration of scientific and technical innovations as well as addressing social considerations to promote the implementation of the principles of circular economy in aquaculture (Aubin et al., 2019; Føre et al., 2018). Seafood has positive health attributes, such as reduced cardiovascular disease risk or better neural development during childhood (Boase et al., 2019; Menozzi et al., 2020; Willett et al., 2019). Thus, especially the health benefits seem to have a high influence on the purchasing behaviour of the consumer (Hoque and Alam, 2020; Pulcini et al., 2020; Wongprawmas et al., 2022).

However, the general perception of aquaculture products in most European countries tends to be negative, e.g., due to concerns about animal welfare, potential climate impacts on aquaculture production, as well as general sustainability characteristics (Alexander et al., 2016; Claret et al., 2016; Feucht et al., 2018; Hoerterer et al., 2022a; Wong- prawmas et al., 2022). This negative perception is believed to have the greatest impact on the low consumption rates of farmed fish in Europe and can be mainly linked to false and misleading information in the media (Govaerts, 2021; Heide and Olsen, 2017; Kreˇsi´c et al., 2020). This misrepresentation of aquaculture and the resulting knowledge gaps, especially in Europe, leads to a prevailing overall negative perception of farmed seafood (Froehlich et al., 2018; Hoque and Alam, 2020; Thomas et al., 2018) and leads to low consumption rates of aquaculture products in Europe (Banovic et al., 2019; Boase et al., 2019; Gaviglio, 2009; Menozzi et al., 2020; Schlag, 2013). The literature suggests that the key drivers of seafood consumption are demographics, knowledge, and personal background (traditions, culture, etc.) (Boase et al., 2019; Verbeke et al., 2007; Vinayak and Arora, 2018). These need to be considered when promoting sustainable eco-intensification of aquaculture production, given the social boundaries closely linked to the labour market and public health (Gerten et al., 2020; Raworth, 2017). Thus, the only thing that can be actively changed in a society is to increase awareness and knowledge, which is why it is crucial to understand the level of knowledge on the individual consumer level (Boase et al., 2019; Kreˇsi´c et al., 2020; Wongprawmas et al., 2022).

In light that consumers often have limited knowledge about aqua- culture in general, it is assumed that positive attitudes, shaped by improved scientific knowledge towards seafood, can increase its consumption and thus influence the overall sustainability of this sector (Kreˇsi´c et al., 2020). Therefore, the increase in consumption and awareness is related to companies placing more importance on and promoting of these factors. However, despite increasing awareness and campaigns aimed at changing consumer habits towards buying sustainable aquaculture products, overall consumer and Yang, 2011). Indeed, to find an influential market strategy, it is crucial to understand consumer preferences, perceptions and choices (Menozzi et al., 2020; Hoque and Alam, 2020). Under this premise, the EU Horizon 2020 project GAIN (Green aquaculture intensification in Europe funding N° 773,330) commenced in 2018. This project has a strong focus on investigating the potential of ecological intensification of European aquaculture. Key elements of this project are feed improvements, circular economy, precision aquaculture, policy, and markets. Drawing on a transdisciplinary approach, research efforts are focused on integrating scientific and technical in- novations, new policy and economic instruments, and reducing social constraints, i.e., identifying and addressing the prevalence of negative perceptions of aquaculture (https://www.gain2020.com).

Within the framework of the GAIN project, this study investigated:
Research question (RQ 1): What role do cultural preferences of individual countries play in consumer choices?
RQ 2: Does more scientific knowledge about seafood products lead to increased consumer awareness and thus hosts the potential to positively change purchasing behaviour towards sustainable aquaculture products? More specifically, does improved scientific knowledge transfer in different European Union countries has the same effect on the purchase of aquaculture seafood products?
RQ 3: How can we positively nudge the consumers perception and acceptance towards more sustainable aquaculture seafood products?
To address these somewhat intangible research questions and especially the role of scientific information as baseline for individual decision-making therein, a novel mixed-method approach is required. This is elaborated on more detail in the next section.

2. Material and methods

This study is the first to look specifically at the role of scientific knowledge rather than general consumer awareness at large. To this end, an interactive poster study approach was selected in order to better exchange with participants and understand their motivations within a rather informal setting. Such interactive poster studies are still rarely used, and more investigations about them are needed to draw better conclusions about their practicability. As such, this is the first study of its kind and is primarily intended to uncover initial trends rather than statistically relevant data, which would be needed in subsequent studies to fully validate these primarily findings obtained here.
Based on an extensive literature search, we identified several criteria that have the biggest influence on the individual purchase decision: price, health benefits, importance of certification, animal welfare, and product processing (Cantillo et al., 2021; Kreˇsi´c et al., 2020; Menozzi et al., 2020; Pulcini et al., 2020). We decided to focus on these central five criteria in order to make the posters as simple and accessible as possible vis á vis to obtain first insight into the differences concerning the magnitude of available scientific knowledge and its respective influence on individual decision-making. Employing a mixed-method approach (Danermark et al., 2019; Kelle, 2014; Levitt et al., 2018), the selected purchase criteria were quantitatively assessed via an interactive poster survey, followed by a series of semi-structured interviews for descriptive refinement and validation (Dale and Kline, 2017; Esterberg, 2002; Mabrouk et al., 2018; Rowe and Ilic, 2009). This makes this approach particularly effective (Diebold et al., 2017; Michalsky et al., 2018). All methods were pre-tested, and the outcomes of the first surveys were choices of seafood are still poorly understood and highly complex (Almeida et al., 2015; Hughes and Black, 2016; Moschitz et al., 2021; Solgaard
further refined.

Poster surveys

In order to clarify the specific national context of consumers, we conducted a series of interactive poster surveys to validate findings from collated secondary data sources (FAO country statistics and Eurobarometer). This method is highly suitable if unbiased data is needed on a specific topic (Pratt et al., 2000; Salzl et al., 2008; Hilton, 2015). However, a prerequisite for the application is to allow enough space for responses (which we ensured by the subsequently conducted semi-structured interviews) and not to present too many details (Altintzo- glou et al., 2018). Furthermore, the format must be clear from the onset and must include the thematic focus of interest. In our case, the focus was 1) to gain an understanding of consumption preferences of aquaculture products and 2) on the importance of individual viewpoints on eco-intensification measures concerning purchase criteria with regard to different scientific knowledge degrees. In this way, it is possible to estimate how much scientific knowledge, expectations, and assumptions participants have about aquaculture at the individual level as a first approximation. Thereby, we follow Esterberg's call (2002) for knowledge exchange as a relationship between two individuals who come together "to try to create meaning about a particular topic," thereby drawing on a range of different social conventions and cultural knowledge. In this regard, an interactive poster survey is more open, easier accessible and conversational than a structured interview or questionnaire.

Pre-test

The first draft of the poster survey was pre-tested (Pratt et al., 2000; Altintzoglou et al., 2018; Ikart, 2019) at a non-coastal (inland) research institution (Germany) to get a better idea of the perceptions of laypeople that have no professional relationship with aquaculture. Thus, a limited degree of scientific knowledge of aquaculture can be assumed. This pretest revealed that it is essential to add the option "not eating seafood at all" to the Poster inquiry, as well as to provide room to enquire about other marine species. The pre-test also revealed the problem of language and understanding of the questions, which was modified towards more simple language use in the poster surveys.

Poster survey at knowledge transfer events

Interactive posters are an effective way to engage, communicate, and share information with attendees of all types (Dale and Kline, 2017; Michalsky et al., 2018). In this study, we used this type of poster surveys at different scientific venues as a validation method, which has been shown to be an effective tool when combined with semi-structured interviews (Hulland et al., 2018). Surprisingly, very few studies explore an interactive approach, despite that posters are standard tools at scientific conferences (Diebold et al., 2017; Mabrouk and Schelble, 2018). In addition, previous attempts to motivate scientists to conduct online surveys at conferences are highly challenging (see Hoerterer et al., 2022b). This can be related to the observation that people are rather unwilling to undergo an online survey whilst being more motivated to look at posters and indicate their preferences briefly in an easily accessible manner. The poster survey aimed to quantitatively identify the prevalent purchase criteria and species preferences (Annex 1). The presented species ranged from marine Atlantic salmon (*Salmo salar*) and sea bream (*Sparus aurata*) to freshwater rainbow trout (*Oncorhynchus mykiss*) since these are the most commonly cultured finfish in terms of value and production volume in Europe (EUMOFA, 2019a; FAO, 2018). In the EU-GAIN project, these three species were the central candidates for circular economy approaches in aquaculture (Hoerterer et al., 2022a; Petereit et al., 2022 in preparation). However, for the poster survey in Spain, trout was exchanged with the European seabass (*Dicentrarchus labrax*) since this species is much more common in southern Europe and was a further central species for the feed experiments in GAIN (Hoerterer et al., 2022a; Muñoz-Lechuga et al., 2018; Petereit et al., 2022 in manus.). To clarify the specific national context of consumers, the poster surveys were conducted in different countries employing predetermined questions (PDQ's) as well as one open-ended question (OEQ). The data were collected at eight different knowledge transfer events that were In total, more than 400 participants took part in the two-page poster survey. The participants consisted of scientists with or without an aquaculture background, aquaculture operators, students, and laypeople across different age groups and with varying levels of scientific knowledge about seafood products. For the latter, detailed online surveys were conducted, and findings were collated and analysed in Hoerterer et al. (2022b). Of these, 383 filled in the more detailed questions on seafood purchasing criteria, next to the questions on species preferences. From these 383 participants we randomly picked some and conducted short semi-structured interview to validate their choices stated in the poster survey (See 2.2). In order to provide a "low-barrier- to-entry" situation, all posters were placed in a public spot where people were able to pass by frequently in a relatively informal manner. This methodological approach has already been tested in various studies and found to be useful in practice (Dale and Kline, 2017; Mabrouk et al., 2018; Michalsky et al., 2018). The results were validated with findings from secondary data sources (Eurobarometer, 2018; FAO, 2020). All responses were self-administered by the participants (i.e., participants completed the survey themselves without interference from the researchers). The subsequent semi-structured interviews took place after the participant finished his/her preference indication on the poster survey. Therefore, the resulting dataset contained fully anonymous and non-identifiable records.

Semi-structured interviews

To validate the poster survey results and obtain more in-depth knowledge, around 40 semi-structured interviews were conducted alongside the poster at the various knowledge transfer events. Semi-structured interviews are a commonly used method to achieve a deeper understanding of human experiences (Bearman, 2019; Dale and Kline, 2017). These semi-structured interviews helped interpret the selections made on the poster, clarified the results, and, by definition, took place in an informal tone and were used to gain more insight into the participant's disclosures (Longhurst, 2004). Recording of the interviews was not conducted as the central purpose of these was to gain more insight into the purchase decision on an individual level and to keep the validation process as unintrusive as possible. In these interviews, participants were asked to justify and explain their decisions in more detail. These were conducted randomly with participants at scientific conferences and similar knowledge transfer events, at which they indicated their consumer preferences via the poster survey. Despite the limitations in regard to representativeness, these provide good insight into the decision-making process of the individual consumer and the role scientific knowledge plays therein.

Location, estimated total number of participants at the different knowledge transfer events (KTE), no. of participants that engaged with the poster, categorization of participants and respective level of scientific knowledge of the eight KTE.

Country	Total participants	Poster participants	Category	Level of scientific knowledge
Germany	250	113	Young marine scientists	Low
Belgium	Unknown	15	Administrative staff of the EU	Low
Germany	50	26	Marine scientists and technicians	Medium
Spain	150	63	Marine scientists and administrative staff	Medium
Germany	50	35	Seafood specialists	High
Germany	50	27	Fisheries scientists	High
Germany	2700	52	Aquaculture scientists	High
Poland	250	52	Carp aquaculture specialists	High

Table 1 conducted under the umbrella of GAIN outreach activities. These took place between October 2019 and February 2020 in Germany (*n* 5), Belgium (*n* 1), Spain (n 1) and Poland (n 1) (Table 1).

Description of scientific knowledge transfer events, their focus, and participants' characteristics

We conducted the interactive poster surveys and semi- structured interviews at eight different scientific knowledge transfer events (KTE), addressing respective target groups and various degrees of participation rates (Table 1). In order to categorize collected data by the level of scientific knowledge, we classified the participants of the respective KTE into low, medium, and high knowledge groups after the semi- structured interviews during the survey. This was done out of the recognition that the different KTEs were targeting different audiences with varying degrees of scientific knowledge on aquaculture.

These different levels of scientific knowledge surfaced during the poster survey and subsequent semi-structured interviews that allowed categorization of their knowledge base. For instance, the group of "low" scientific knowledge consists mainly of younger participants (e.g., mostly Generation Z and a few Generation Y's) with only a very marginal background in the topic of aquaculture research. This group mainly got their aquaculture knowledge from the media and displayed in general very negative and fixed mindsets. The second "low scientific knowledge" category consisted of educated laypeople that were located inland in a major city that showed an overall low knowledge about different practices and sustainability of aquaculture. In contrast, the "medium" knowledge groups consist of people who stated to have some degree of exposure and knowledge of aquaculture. This category consists mainly of scientists that are not directly involved in aquaculture research but in other marine biology studies (such as sea shelf ecology, fisheries, or general biology studies). The last category with "high" scientific knowledge were experts with a strong degree of scientific knowledge in aquaculture production or related fields and/or were professionally involved in the seafood production sector. The latter category was the easiest to categorize because the KTE were very specific aquaculture conferences, in which only people within the aquaculture research field were present. The semi-structured interviews revealed here that all participants had an excellent background in scientific knowledge of aquaculture and were e.g. aware of different rearing systems, sustainability issues, and other factors influencing the industry. For most KTE, we were able to estimate the number of participants at the conference by either asking the organizer or our own rough estimations.

Results and discussion

Analysing the consumer decision-making process is rather complex as many different contextual drivers act on the individual seafood preference that varies over time. However, in order to shed some light on the impact of scientific knowledge in relation to purchasing behaviours whilst acknowledging country-specific differences, we first present and discuss the findings on a country level (research question 1), followed by an assessment of the influence of respective scientific knowledge levels (research question 2), and closing with an individual-level analysis (research question 3). Rooted in the findings of this multi-level and multi-dimensional analysis, recommendations for future research needs and the prospects of seafood preferences are outlined.

Country-specific differences

To gain insights on the question of whether there are potential country-specific preferences in seafood consumption, the poster surveys were conducted at different knowledge transfer events (KTE) per country (Research question 1). These ranged from Spain as a country with one of the highest per capita consumptions with over 45 Kg live weight/ capita/ year, to Belgium, Germany, and Poland as European countries with decreasingly lower per capita consumption rates (13,5–15 Kg/capita/year) (EUMOFA, 2019b). Participants from six out

Table 2

Species preference according to the different nationalities.

Overall Participation	Germany	Spain	Poland	International*	Total
	181	117	54	83	435
Salmon	37%	17%	28%	42%	31%
Sea Bream	20%	12%	2%	37%	19%
Trout	14%	1%	26%	17%	13%
Seabass	1%	20%	4%	0%	6%
Other	8%	50%	30%	0%	21%
No seafood consumption	18%	0%	12%	3%	10%

* International includes the participants from knowledge transfer event 2 ($n = 15$) and from 7 ($n = 52$).

of eight KTE were explicitly conducted in the specific countries mentioned above. The other two KTE were conferences that had mixed international participation and thus were not assigned to a single country (KTEs 2 and 7). In the following subsections, the results of the various country-specific aspects of seafood consumption preferences are examined in greater detail. The pre-tests showed that especially the species and food processing could be well mapped between the countries. Therefore, we focus particularly on these aspects, as both species preference and processing were cited as the most important determinants of the purchase decision in the literature and were reinforced in our semi-structured interviews (Cantillo et al., 2021; Krešić et al., 2020; Pulcini et al., 2020.

Finfish species preference per country

All participants (n 383) from the various KTE had a clear preference for salmon (31%), closely followed by sea bream (19%) (Table 2). This is consistent with the results of the Eurobarometer survey, which found that 35% of consumers prefer salmon over other species (EUMOFA, 2019a). One-fifth of participants indicated that they prefer other species than the three main given species and therefore provide information about differences across nationalities (Table 2). In the subsequent semi-structured interviews, the latter participants stated that they do not eat seafood due to vegetarian or vegan dietary preferences. On average, 33% stated never to buy fresh seafood products in our study. These findings are consistent with recent studies that indicate that veganism and vegetarian lifestyles have been growing in recent years (Saari et al., 2021). This vegan/vegetarian movement is often a very individual decision and, more often than not, strongly linked to peer group pressure. However, the understanding of why specific food lifestyles are chosen is not yet well understood. For instance, vegan and vegetarian lifestyles are also known to reduce the incidence of heart disease and diabetes, leading to better health outcomes (Menozzi et al., 2020;Pieniak et al., 2010). In addition to personal preferences, animal welfare and sustainability play a crucial role for the decision to go vegan /vegetarian. Many consumers are, due to various reasons, subject to the prejudice that seafood, in general has a negative impact on the ecosystem, as well as on labour conditions (Govaerts, 2021). These prejudices can be linked to the reinforcement of false media image, that more often than not, remain in the prevailing negative narrative of harmful aquaculture systems, thus ignoring contemporary efforts and achievements made towards sustainable aquaculture production systems. In conclusion, why the vegan lifestyle is increasing and what repercussions this has on the consumption of aquaculture products (e.g. seaweed) is a very challenging issue and needs further investigation.

In comparing the different nationalities, only 8% of German participants stated eating species other than the three listed on the poster survey. That said, up to 68% of all species consumed by German participants can be split between salmon (37%), sea bream (20%) and trout (14%) (Table 2).

However, interestingly roughly 19% of German participants reported never eating seafood, which is in stark contrast to Spain, where none indicated such a (non-)preference. This reinforces previous studies, showing that German consumers are known to be attracted to the fish they are most familiar with (Koch et al., 2019).

In addition, with the exception of salmon, herring and to some extent, seabream, there is very limited advertising in Germany to guide consumers to alternative seafood species. In contrast, Spain has the most diverse species consumption composition, with about 50% indicating to consume none of the three most frequently mentioned seafood species in Europe as a whole, but rather consume a diverse set of other marine species. Contrasting this diverse seafood preference picture in Spain, 30% of seafood consumed in Poland is not covered by the top three preferred marine species in Europe but refers to other, mainly fresh water species, such as perch (31%) and carp (38%). Country-specific differences between the selected EU countries can be further identified by focusing on the distribution of “other” seafood consumed. Participants were asked to indicate seafood species other than the species listed on the poster. For example, in the semi-structured interviews, Spanish participants indicated that they strongly preferred local (marine) species as long as they were caught nearby and thus indicated a strong recognition and motivation to support local fisheries and working communities.

Processing preferences per country

There are considerable differences between the respective EU countries in terms of the degree of consumer preference for processed seafood. Overall, most respondents (65%) indicated to prefer fresh seafood over frozen (17%) and processed products (6%) (Table 3). Our survey results mirror the findings of the Eurobarometer survey (2018) that found similar trends across the EU, where fresh products (37%) are preferred over frozen products (25%). However, the most distinct distribution in preference for processed seafood was found in Poland. Here, 56% of respondents prefer fresh fish over frozen (21%) and processed products (12%), and 12% said they never eat seafood (Table 3). Regarding the consumption of frozen products, all survey participants from Germany, Poland and Spain indicated that they buy such products at least occasionally. However, 76% of all Spanish responders stated to prefer fresh fish over frozen (22%) or processed (2%) fish, and none declared not eat seafood at all. This finding is also reflected in the 2018 Eurobarometer data, which shows that consumers from Spain, followed by Greece, are most likely to buy fresh seafood products (EUMOFA, 2018).

These country-specific differences mirror the respective social-cultural settings in which seafood consumption has been traditionally placed. For example, Spain has a long fishing tradition, which explains the prevalence and acceptance of a wide variety of fish species and the high percentage of consumers who prefer fresh fish. Indeed, traditionally Spanish consumers go to the market and buy fish offered by local fishermen instead of choosing the cheapest processed fish product in the supermarket (Jacobs et al., 2015).

Our surveys reinforce this, whereas 80% of international and Spanish participants indicated that they prefer fresh fish over frozen or processed products. However, these results must be taken with some level of caution due to the low overall number of participation and the fact that all Spanish participants were “economically highly affluent”. Thus, this observed trend in our surveys may not reflect the entire country of Spain. However, it nonetheless can still be reconciled with the results of the semi-structured interviews, where respondents indicated that they strongly support their local fisheries by purchasing seafood products directly from the (local) market.

Differences in the level of scientific knowledge

In the following, we will explore the question of how the degree of available scientific knowledge about seafood products influences consumers purchasing behaviour (Research question 2). Generally, it is assumed that a rise in scientific knowledge about a given product leads to more sustainable purchasing decisions due to the increasing awareness of the problem (Almeida et al., 2015). However, this assumption is difficult to validate due to biased perceptions of the consumers surveyed and categorization of the scientific knowledge itself. Indeed, personal perspective surveys always inherit a factor for error, especially on contested and highly normative topics such as individual food preferences.

Finfish species preference in relation to the degree of scientific knowledge

Drawing on the knowledge transfer events and especially focusing on KTE 1, a conference for young marine scientists with relatively little prior scientific knowledge about seafood production, it is noteworthy that more than one-third emphasized in the semi-structured interviews not eating any seafood or fish at all (37%). Moreover, this was reinforced by the poster survey during this KTE that the participants largely did not indicate any seafood species other than the three suggested on the poster. Those who did indicate consuming seafood stated to prefer salmon (30%), followed by sea bream (18%) and trout (15%). In contrast, the results for the KTE 7, where all survey respondents inherited a high level of scientific knowledge about aquaculture and its products, show only a marginal 5% proportion of non-seafood consumers. Almost half of all participants of the latter KTE stated salmon as their favourite fish species (47%). Sea bream came in second at 32%, followed by trout at 16%.

Most of the participants at the KTE 1 indicated in both survey formats (poster survey as well as subsequent semi-structured interviews) to be highly concerned about healthy lifestyles and the importance of healthy foods in general, which resulted in their decision towards a vegan lifestyle. Indeed, healthy foods are becoming more and more trending, especially in the western world, where healthy diets receive increasing attention among groups with a higher degree of scientific knowledge (Saari et al., 2021; Tomi´c et al., 2017). Long-chain polyunsaturated fatty acids, like Omega-3, are highly present in fish and are very beneficial for human health. Hence, these are advocated by governments worldwide (Tomi´c et al., 2017; Turchini et al., 2011; Verbeke et al., 2007). Despite these widely known facts, most of the young marine scientists participating at the KTE 1 indicated they do not eat seafood despite knowing about its health benefits. These results support the hypothesis that scientific knowledge about seafood products is not necessarily per se a driver of purchases. Rather, purchasing seems to be related to situative individual decisions driven by cultural practices, peer-group pressure, and world views.

Table 3

Processing preferences divided for the different countries.

Overall Participation	Germany	Spain	Poland	International*	Total
	201	63	52	65	381
Fresh	58%	76%	56%	80%	65%
Frozen	18%	22%	21%	8%	17%
Processed	5%	2%	12%	9%	6%
No seafood consumption	18%	0%	12%	3%	12%

* International includes the participants from knowledge transfer event 2 ($n = 15$) and from 7 ($n = 52$).

The role of certification in relation to the degree of scientific knowledge

In most cases, participants' response to whether certification is a purchase criterion was above 50% at all KTE. This indicates that certification as a purchase criterion is important, but not as strongly decisive as commonly believed (Asche and Bronnmann, 2017). However, our results show that at all events where participants inherited a high scientific knowledge, an average of 64% indicated that certification is essential for making a purchasing decision. In contrast, 53% of those with low scientific knowledge and 60% of those with medium scientific knowledge stated that certification is an important aspect of their decision-making. These results show some degree of correlation between the degree of available scientific knowledge and the importance of product certification as a purchase criterion. However, our questionnaire did not consider the differentiation between aquaculture products or wild-capture fish. In our pre-test survey we encountered that many consumers appeared to ignore the type of product (wild-capture or aquaculture), but solely placed their decision making upon the certificates, as they assumed that this indicated a higher degree of sustainability. This links Alfnes et al. (2018) findings that consumers demand traceability. Overall, these results emphasize the connection between higher scientific knowledge and an increase in awareness of the product's sustainability. This can be understood as a central incentive to obtain certification from a producer's point of view and shows the trend of rethinking consumer purchase choices. Indeed, the semi-structured interviews reinforced that most participants were well aware of the MSC logo, while only a few stated to ever have paid attention to its aquaculture counterpart, the ASC logo.

Other purchasing criteria

The remaining criteria listed in our surveys did not show any difference in terms of country-specific or scientific knowledge specific purchasing criteria. For instance, no differences were found between knowledge events regarding animal welfare purchasing criteria (89% overall) and health benefits (64% overall). It is noteworthy that animal welfare was considered very important in all events and across all knowledge levels, nationalities and age groups. Next, all survey results exposed the decisive role of price and origin as purchase preferences. These cannot be related to having distinct country-specific or scientific knowledge availability dimension. Therefore, the following central findings for both criteria are collated.

Price preferences

The price of fish as a purchase criterion is closely linked to the socio-economic positioning of the respective consumer. This affects how much attention is paid to the price of a seafood product. As our surveys exposed, the majority (73%) stated that the price range influenced their purchasing behaviour, and only 29% said they were not concerned about price. The latter is reinforced by earlier findings that show consumers mainly eat fish for its health, nutritional properties, and taste but care less for its origin and more for the price in general (Brunsø et al., 2009; Vanhonacker et al., 2013).

Our surveys revealed that both younger people and noteworthy especially Spanish participants, seem to pay more attention to the price of seafood while the degree of scientific knowledge appears not to affect the overall purchasing decision. In the case of the Spanish consumers, this can be explained by the long fishing tradition in the country, and as Fernández-Polanco and Luna (2012) showed, hence price is the only favourable factor towards purchasing aquaculture products over wild capture-fisheries products. Thus, price perception as purchasing decision factor emerges as the main incentive in regard to fish consumption between countries and user groups with different consumption profiles (Ingram, 2017; SAPEA, 2017). Especially when money is involved, it is a widespread phenomenon across all participants to favour the price above all other criteria. This points to the central problem of willingness-to-pay studies in seafood consumption studies (Chang and Nguyen, 2018; Grunert et al., 2009; Zander and Feucht, 2018). More often than not, studies revealed that individuals state that they ignore the price of a high-quality product (see e.g., van Osch et al., 2017; Xuan and Sandorf, 2020; Yip et al., 2017). However, these statements do not match actual shopping figures from supermarkets (in Germany and elsewhere), which show that consumers, to a considerable extent, tend to buy the cheapest product on the shelf without considering its origin or quality (Eurobarometer, 2018; NSC, 2019). In conclusion, in our survey we could neither see country nor scientific knowledge related differences concerning the price as purchasing criterion for seafood products.

Origin preferences

More than 70% of all respondents to our surveys have begun to consider the origin of the fish. In the semi-structured interviews, particularly with participants with a higher level of scientific knowledge, it was stated that these were more selective with respect to the origin of the seafood product purchased. The interviews further revealed that these consumer groups tend to pay more attention to the regionality of the product, which plays an essential role in the concern of supporting sustainability. This mirrors the findings by Guillen et al. (2019), who demonstrated that competing for resources is a rising issue in finfish production for consumption, and more consumers are willing to emphasize their awareness of sustainability by focusing on the origin of the product. Aquaculture is known to provide a more efficient production system than wild-capture finfish, as well as to be far more sustainable, primarily when a circular economy approach is applied (Regueiro et al., 2021). Our study showed that it can be assumed that more focus on the regionality dimension of aquaculture could improve the demand for aquaculture products, especially if these are produced as part of a circular economy set up. However, consumer concerns and assumptions about regionality and sustainability need to be considered in the promotion and/or production of cultivated fish.

Rethinking the pathways to sustainable seafood consumption in Europe

Today's societies have evolved into multi-layered, highly fragmented and diverse entities with a wide range of interests, views, knowledge structures, perspectives, norms and values (Jacobs et al., 2015). These are also mirrored in the decision-making process of seafood purchasing. This makes addressing our last research question challenging, that is how we can shift consumers behaviour towards a more sustainable purchasing behaviour. To this end, the social dimensions of consumer acceptance and their related individual purchasing decisions must be tackled.

Our study reinforces findings from previous research (such as Asche and Bronnmann, 2017; Fernández-Polanco and Luna, 2012; Karnad et al., 2021), which showed that the degree of scientific knowledge about seafood products does not automatically influence purchasing behaviour. For instance, Fernández-Polanco and Luna (2012) identified the socio- demographic background, product promotion, and price as the three most crucial seafood purchase criteria. Our results are consistent with their findings in that regard that price appears to be the most important purchase criterion across all countries investigated. If we conclude that increased knowledge does not necessarily lead to more aquaculture products being purchased, the question remains as to what would support a change towards more sustainability-oriented purchasing behaviour. The results of the semi-structured interviews conducted in this study revealed that individuals with a high level of scientific knowledge about seafood products are highly interested in the sustainability of the fish they consume. However, this increased awareness does not necessarily translate into direct behaviour patterns in the supermarket when these individuals purchase fish for personal consumption (Lawley et al., 2019). Furthermore, the current multitude of different certification systems certified product labelling often does not clearly identify sustainability aspects hence causing confusion among consumers. Indeed, the wide range of certification labels leads to misunderstandings, and people eventually resign themselves to trying harder to understand the label (Alfnes et al., 2018). Interestingly, the results of our semi-structured interviews indicated that the more people know about a respective certification system, the less they stated to trust it. However, people who are less scientifically knowledgeable about seafood production stated to trust some labels and indicated to become more confused when shopping at the supermarket. One participant at the KTE 1 described feeling that there is an “ecolabel jungle”, with more and more ecolabels popping up almost weekly, promising to be more sustainable and environmentally friendly than the others. In this regard our semi-structured interviews exposed a major problem in purchasing in regard to transparency and traceability of the product. These issues tend to be overlooked and overseen by the producers of aquaculture products but play an important role for the individual purchasing behaviour disregarding the country-specific context nor the degree of available scientific knowledge. Thus, the hypothesis that more scientific knowledge leads to more sophisticated purchasing criteria may be appealing from the onset, but limitations emerge when applying this hypothesis to real-world contexts. The issues of food labelling and confusion in respect to how products are certified reduce the actual intended outcome towards more sustainability-lead purchasing behaviour are a case in point (Ihemezie et al., 2018). The question of how to overcome this problem remains, and more studies are needed that focus on a different way of how to nudge and change purchasing behaviour towards a more sustainable product choice. These must be tailored to country-specific settings and to their respecting processing preferences. The results of this study hence call into question current strategies for promoting sustainable aquaculture products. The more different pro- motional options are offered, the more consumers appear to become confused and eventually resign from the effort to consider sustainability aspects of the product in their purchasing decisions (Ihemezie et al., 2018; Maesano et al., 2019). To overcome this barrier, one option could be to develop a coherent national (or even international) strategy for seafood marketing and more specifically, for sustainable aquaculture. Apparently, as indicated by our results, it is essential to develop a non- confusing option that helps consumers understand sustainability without further confusion. A potential route is the “Nutri Score” developed by the Federal Ministry of Food and Agriculture in Germany. On a range between 1 and 5 the score indicates the nutritional value for each product (Schlögl, 2020). Labelling the product with the score is voluntary, and manufacturers can decide whether or not to use it. Despite that this may not be the perfect solution and is yet contested, it provides consumers more detailed information about the food and nutritional values at first glance in a straightforward manner. A similar labelling system for seafood products, where the sustainability of the product as well as the production circumstances are easier to capture could possibly be a more effective support towards sustainability-led purchasing decisions than 100 different ecolabels. This is mirrored by Maesano et al. (2019) who provided evidence in their literature review that people generally have a higher willingness-to-pay for a product even when they do not fully understand the label but assume that it is a sustainably produced product.

In addition, the current focus on circular economy approaches is another avenue to promote more sustainable and transparent products. Under this umbrella, the aquaculture sector can be assumed to be more economically competitive, if it allows better circular options of pro- duction. In this way, wild fish stocks can be conserved while ecosystem services are improved, leading to better overall consumer perception (Ruiz-Salmón et al., 2020).

Limitations of the study methodology

This study was conducted as part of the GAIN project and serves as the first entry point to understand the role of scientific knowledge and related consumer purchasing behaviour. To that end, it focuses exclusively on the level of scientific knowledge on seafood products, particularly on sustainable aquaculture products, and how specific levels of available scientific knowledge influences consumers purchasing decisions.

However, all social science surveys addressing personal perspectives inherit a factor of error, especially when enquiring into rather normative reasoning of individual understanding of sustainability that can be mostly captured only in a qualitative manner. The distorted perception of sustainability on the individual level as well as the rather coarse categorization of the scientific knowledge levels per individual participant leads to limitations in the scientific rigor of the findings, next to the fact that many participants are not fully honest in their statements made (Bursztyn et al., 2019; Solgaard and Yang, 2011; Zander and Feucht, 2018).

That said, our experience showed that the information captured on the poster survey did not always reflect the perspectives voiced in the subsequent semi-structured interviews. In this regard, the effect of peer pressure (Shu, 2018; Vinayak and Arora, 2018) is particularly note- worthy and surfaced strongly the more people gathered together in small groups to view and complete the poster survey at the same time. A good example of the emergence of this type of peer pressure was in closed events, such as KTE 3, where participants knew each other well and were asked their opinion on the role of price in their purchasing decision. On these occasions, roughly 81% stated to ignore price and focus on other (mainly sustainability-led) buying criteria. This can be explained by the influence of peer group pressure, as Vinayak and Arora (2018) have shown. It can be expected that participants at these particular KTEs do pay attention to price but were embarrassed to indicate it openly among their peers. For instance, the participants at KTE 3 all had intermediate scientific knowledge about seafood and were well aware of the adverse effects of looking only at the price as purchasing decision.

Another limitation is that consumers often misjudge how much they know and do not know about a given subject (Krešić et al., 2020). In our semi-structured interviews, participants often initially claimed to know about the sustainability of seafood products, especially on aquaculture products. However, in the course of the interviews, more often than not the claimed knowledge surfaced to be erroneous when inquiring in more detail about specific sustainability attributes. This overestimation of own (scientific) knowledge often leads to contestation of new knowledge rooted in scientific evidence. Many of the participants interviewed were reluctant to learn about new evidence about sustainable aquaculture, thus showing signs of being conditioned to be highly sceptical of the information they receive resulting in preferring to remain in well-trained purchasing patterns. This somewhat is in contrast to the yet prevailing view that finding scientific facts informs actions. Investigating this discrepancy and the very implications for seafood consumption appears to be a crucial under-researched issue. Further studies need to show the extent to which this knowledge gap impacts consumption.

Future recommendations

Looking critically into the future, we see that Generation Z, born in the digital age between 1995 and the early 2000s, is to date the largest consumer group in the world (Su et al., 2019; Zuo et al., 2022). This generation places a high emphasis on environmentally-friendly foods, sustainability and animal welfare (Su et al., 2019; Zuo et al., 2022). Therefore, the generational differences in purchasing behaviour need to be considered in future campaigns (Kamenidou et al., 2020).

That being said, do most campaigns in the European union do neither focus on generational nor country-specific differences. This strategy needs to be challenged if more awareness and promotion with regard to the sustainability of purchasing decisions of seafood products, specifically aquaculture products, is the goal. A first approach could be to show (especially for Generations Z and Y) the “new” production system, the so-called circular economy approaches. Studies have shown that the younger generation is especially interested in sustainability (carbon footprint) and upcycling of food to avoid food crises and waste (Kymäänen et al., 2021; Zhang et al., 2021).

Another aspect for future studies is the application of interactive posters surveys, which is method still very underutilized in the field of aquaculture and consumer perceptions research. This study demonstrated an initial approach to incorporating interactive poster surveys at conferences to gain valuable insights into attendee perspectives. We would recommend using these interactive posters in awareness campaigns to better understand the rather intangible reasoning of consumers and their purchasing decisions, vis á vis to spark their interest in aquaculture products. A study by Michalsky et al. (2018) examined an interactive approach to changing attitudes towards people living in poverty. The study used social media and interactive installations to “put the user in the shoes of someone living in poverty.” The interactive engagement of participants generated an emotional response and led to greater understanding. It also fostered a relationship between people experiencing poverty and the study participants. Using this study as a model, we would make a similar recommendation for the future. For instance, one approach could be to show seafood buyers the different living conditions of a fish in the wild and a fish in aquaculture, focussing on the health benefits of farmed animals (fewer toxins, fewer sick animals), sustainability (no bycatch, usually poor working conditions in fisheries), and reuse of resources (circular economy). Such an approach may potentially foster a similar emotional response from participants, leading to a change in purchasing behaviour.

Conclusion

Preferences are highly related to a rather intangible set of individual cultural context-dependent drivers that somewhat contradict the affirmative role of scientific knowledge in a healthy information society. To this end, we are aware that this study provides only a first impression on this subject and further and more detailed research is needed to prove the here stated results. In conclusion, the rapid changes in consumer demographic, social, and economic structures and production patterns call for new research on translating information and awareness into practice. Our study demonstrated that consumers' beliefs, norms and values all effect personal perceptions and consumption of aquaculture products. These often appear to be more important than other categories such as animal welfare, price, or origin. Perceptions and purchasing habits are dynamic and vary from culture to culture. A lack of clear and accessible information can generally be considered the main barrier to the social acceptance of aquaculture products in Europe. Potential country specific differences in species composition preference could be identified. In contrast, results on the role of scientific knowledge were rather blurred. Some participants in this study exhibited a high level of scientific knowledge about aquaculture products while still choosing the less sustainable product option due to financial reasons. This indicates that price appears to be the most important purchasing criteria. Next, we found that younger people with less scientific knowledge about seafood were more likely to adopt a vegan/vegetarian lifestyle.

We conclude that food and, more specifically, country- specific food culture plays an important societal role. By understanding consumer preferences in different EU countries and using diverse scientific evidence, opportunities to transform current marine food systems in the EU may emerge.

However, more data are needed to provide further information on the relationship between scientific knowledge, food culture and respective purchasing behaviour. Our findings raise the question whether trying to educate people about more sustainable purchasing criteria is really the key to more sustainable purchasing or are we, based on wrong assumptions, applying wrong approaches to foster more sustainability-led purchasing decisions.

CRediT authorship contribution statement

J. Petereit: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization.
C. Hoerterer: Conceptualization, Methodology, Validation, Investigation, Writing – review & editing.
Krause: Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Fund- ing acquisition.

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.

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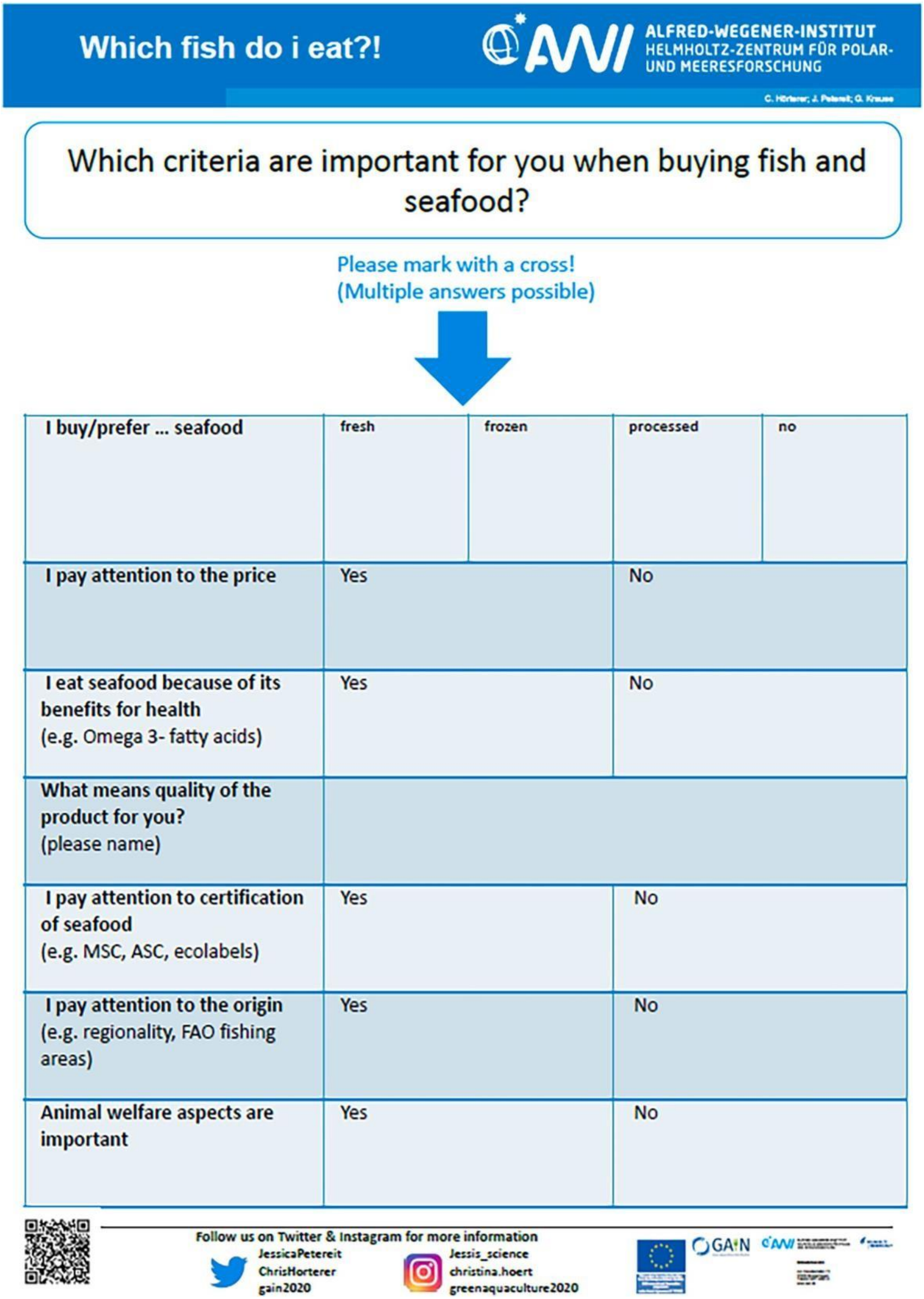

Which fish do i eat?!

ALFRED-WEGENER-INSTITUT
HELMHOLTZ-ZENTRUM FÜR POLAR- UND MEERESFORSCHUNG

C. Hörterer; J. Petereit; G. Krause

Which criteria are important for you when buying fish and seafood?

Please mark with a cross!
(Multiple answers possible)

I buy/prefer ... seafood	fresh	frozen	processed	no
I pay attention to the price	Yes		No	
I eat seafood because of its benefits for health (e.g. Omega 3- fatty acids)	Yes		No	
What means quality of the product for you? (please name)				
I pay attention to certification of seafood (e.g. MSC, ASC, ecolabels)	Yes		No	
I pay attention to the origin (e.g. regionality, FAO fishing areas)	Yes		No	
Animal welfare aspects are important	Yes		No	

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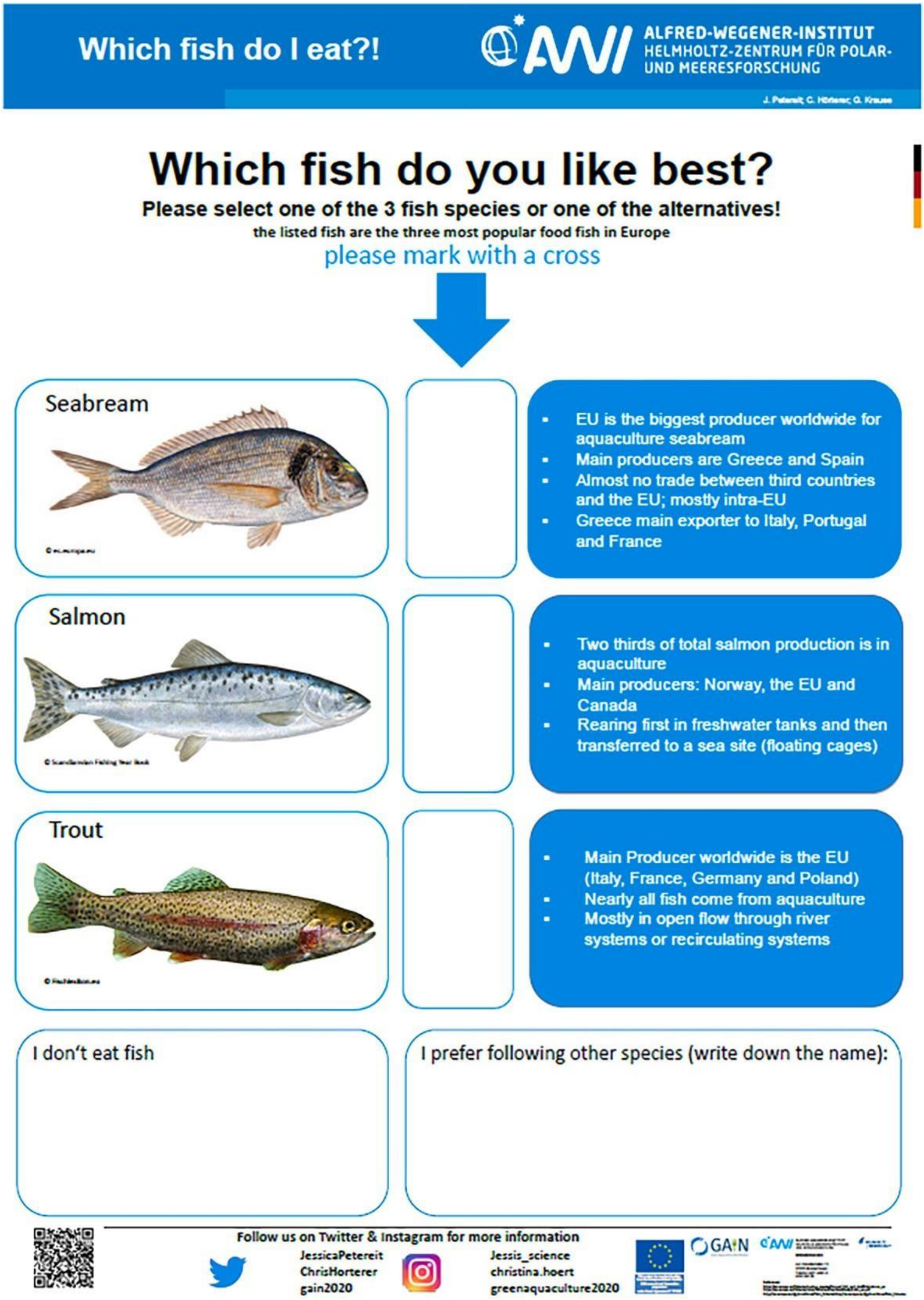
Annex 1. Purchasing criteria poster.



Annex 2. "Which fish do you like best", species preference poster.

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Chapter IV

Adult European Seabass (*Dicentrarchus labrax*) Perform Well on Alternative Circular-Economy-Driven Feed Formulations

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Article

Adult European Seabass (*Dicentrarchus labrax*) Perform Well on Alternative Circular-Economy-Driven Feed Formulations

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Abstract: There is an increasing need in the aquaculture industry for more sustainable and functional feed concepts for marine finfish. This study provides results for the effect of alternative feed formulations on health status, welfare parameters, sensory analysis, and growth performance in European seabass (*Dicentrarchus labrax*) over an 83-day feeding trial. Fish were fed twice a day with five experimental diets. A control diet (control) and four different alternative feed concepts rich in processed animal proteins (PAP), other alternative ingredients (NOPAP), and a positive (NOPAP$^+$) and negative (PAP$^-$) formulation were tested. All alternative formulations contained hydrolysates from aquaculture by-products and macroalgae. The results indicate that the alternative feed concepts are more sustainable alternatives compared with the commercial diet. Equally interesting, the alternative formulations did not affect the sensory analysis of the fillet quality or the animal welfare. These are increasingly important factors in aquaculture products and, accordingly, also in the formulation of new feeds. Feed concepts that are not only more sustainable in their production, have shorter transportation distances, recycle the resources (usage of by-products), and have no adverse effect on growth or welfare parameters are highly needed. Therefore, the experimental diets tested in this study are a win-win concept for future seabass aquaculture production.

Keywords: insect meal; by-products; sustainable feed; fish welfare

Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), more than 10% of the human population suffered from hunger in 2018, with increasing numbers over the last decade [1]. Therefore, the demand for protein sources to meet world hunger and nutritional requirements is rising. Aquaculture, as the fastest-growing, food sector holds promise for food security and offering new job opportunities [2,3]. Furthermore, it already accounts for 52% of global seafood consumption and can provide a more sustainable alternative than commercial fisheries [2].

To optimize aquaculture production while improving sustainability and ensuring the nutritional status of the fish for human consumption, it is crucial to work towards high quality, eco-friendly, and affordable feed concepts [4]. Sustainability in this paper refers to a more circular economy process that focuses on ecosystem conservation while ensuring excellent nutritional and welfare parameters for the fish [5]. The circular economy approach is the most promising and widely used model in aquaculture. It involves the reduction and reuse of resources in production while aiming for optimization and eco- efficiency [6]. The main sustainability issue in aquaculture is the fish in/fish out ratio, which needs to be addressed to ensure a more sustainable production while minimizing resource consumption and maximizing yield [5].

Feed represents the main operational cost in marine fish farming. At present, the main protein sources are fishmeal (FM), soybean protein concentrate (SPC), wheat/corn gluten meals (WGM/CGM), and soybean meal (SBM) [7-9]. The FM, SPC, and SBM need to be augmented with alternative sources due to their high price and questionable sustainability to ensure the growth of aquaculture production [9,10]. FM is regarded as unsustainable due to finite marine resources, long transportation distances, as well as habitat degradation, and needs to be replaced to improve the sustainability of fish farming [10]. SBM is mostly produced in the American continent, in countries that often grow soy in unsustainable monocultures, which not only challenges the availability of land for food production, but also produces high transportation costs to get the meal to Europe, increasing the carbon footprint for the feed [11].

The demand for high-quality proteins in aquaculture feeds is high, and sustainable alternatives, such as plant- and terrestrial-animal- derived proteins are needed. In addition, protein hydrolysates produced from aquaculture by-products can help ensure the food safety and nutritional balance of fish while eliminating the independence of import and long transportation ways [8]. This is especially important in current situations that increase the demand for independency in the context of global pandemics (e.g., COVID-19 or other diseases), political escalations (e.g., wars or other forms of conflicts), or natural phenomena and disasters (El Nino, tsunamis, etc.) that lead to transportation bottlenecks [12]. The definition of by-products includes all raw materials (edible or inedible) that are left over from the production of the main product (here: seafood in general) and can be directly reused without further processing [5,6]. These include waste, skins, heads, blood, and bones. In fish production, an estimated 45% is used as a primary product and 55% as a by-product, highlighting the need to reuse these products to reduce environmental impact (carbon footprint with longer transportation distances) and improve production efficiency [6].

Other alternative protein sources are insect meals, plant-derived proteins, terrestrial animal by-products, fermented biomasses, and/or microalgae [10]. Taurine is deficient in plant-based protein sources, which is known to be essential for growth in carnivorous fish, such as European seabass or turbot [13,14]. It is well known that plant-derived proteins lack the nutritional benefits for feed performance in carnivorous fish and can only account for a smaller proportion in fish feed [15,16]. Simple-stomach animals, such as carnivorous fish, cannot digest plant ingredients, such as fiber. The cellulose in the feed leads to reduced energy, protein, mineral, and nutrient availability for the fish, as the complex cell walls cannot be digested [17]. Currently, we can see a steady increase in the formulated fish feed based on a mix between FM, aquaculture by-products, plant, and animal-derived proteins that lead to better digestibility of the nutrients for the fish [6,13].

Another rising issue is the mineral deficiency in fish caused by unbalanced diets that limit the mineral availability [18]. This can lead to a poor nutritional quality of the final products, so it is essential to evaluate a fish species' mineral and trace elements when looking at alternative feed ingredients [19].

It is known that carnivorous fish, such as European seabass, are hampered in the extraction of macronutrients from plants/algae [20]. This reduced availability of nutrients can cause harm in the intestinal tracts and could lead to inflammation and poor health, which is why the apparent availability needs to be considered when looking at alternative feed diets [20]. Phosphorus, calcium, iron, zinc, and iodine are the most nutritionally essential elements in marine fish, with total mineral content ranging from 0.6% to 1.5% on fresh weight [18,21]. The skeletal structure, the preservation of the colloidal system, and the regulation of acid–base equilibrium are all critical activities of vital minerals, which are absorbed from the food and deposited in skeletal tissue and organs [21,22]. In general, fish from aquaculture have fewer toxic elements in the final products than wild fish, because wild fish bioaccumulate hazardous substances from the sea. This is another reason why aquaculture fish from RAS are a more promising solution for the safe consumption of seafood [6,22]. Welfare assessment in the aquaculture sector is becoming increasingly important for both consumers and governments, and evaluating new feed concepts in that aspect is mandatory for sustainable development [23]. For welfare assessment, it is essential to have a multilevel approach, as individual parameters are not giving an accurate evaluation of the complete state of the fish [24]. The welfare of fish is a highly complex system. The literature suggests using stress responses to assess the animals' physiological status [24-27]. Therefore, this study investigates blood parameters and immunological responses (lysozyme), known as key stress parameters [24,25,27]. European seabass is a marine fish species that is highly important in Europe for cultural as well as economic value [28,29]. As a carnivorous aquaculture species, it relies on high protein feed ingredients, usually FM, in the feed to ensure good health and growth performances [30]. It is known from the literature that FM in sea bass can be partially replaced by insect meal and plant and animal proteins without affecting the metabolism and/or growth [17,28,31,32].

This study focused not on a single ingredient analysis, but rather feed formulation concepts based on combinations of more sustainable ingredients to test for growth, nutritional, welfare, and sensory parameters. Four alternative diet compositions were tested against a commercially mimicked feed recipe to offer more sustainable feed formulations for the seabass farming industry. Feed intake, growth, feed utilization, welfare parameters, tissue composition, sensory, and mineral analysis were assessed over an 83-day feeding period.

Materials and Methods

Experimental Setup

A total of 375 European seabass (*Dicentrarchus labrax)* kept in the recirculation aquaculture system (RAS) of the Alfred Wegener Institute Helmholtz Center for Polar and Marine Research (AWI) (Bremerhaven, Germany) were used for the feeding experiment. The fish were acclimatized within the RAS for two weeks before starting the 83-day feeding trial. Fish were individually tagged with PIT tags and, afterwards, randomly distributed into 15 tanks (25 fish/tank) for acclimatization (Tag reader: Agrident; APR600 ISO 11784/11785 RFID Handheld Reader). The mean weight of the individuals was 320.8 ± 72.4 g. and the mean length was measured at 30.5 ± 2.1 cm, respectively. The experimental RAS consisted of 36 individual holding tanks, with a bottom area of one m^2 and a volume of 700 L each. For all 36 tanks, the water was treated the same, using typical RAS cleaning devices, such as a drum filter, bio filter, a protein skimmer (with ozone), and a trickling filter. For this experiment, 15 out of the 36 tanks were used to ensure a density comparable to aquaculture facilities. The rest of the tanks were used as holding tanks for the spare fish. The condition (temperature, pH, salinity, and oxygen) of the process water was monitored daily with an SC 1000 Multiparameter Universal Controller (Hach Lange GmbH, Düsseldorf, Germany), and the nutrient concentration (nitrite, nitrate, and ammonium) was measured with the QuAAtro39 AutoAnalyzer (SEAL Analytical, Germany) twice a week (Table 1).

Table 1. Mean values ± standard deviation of the water parameters during the experimental trial (83 days) (temperature, pH,salinity, and oxygen: n = 83; ammonium, nitrite, and nitrate: n = 42).

Temperature (°C)	pH	Salinity	Oxygen (%)	Ammonium (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)
20.0 ± 0.9	7.62 ± 0.04	36.3 ± 0.1	95.2 ± 6.7	0.096 ± 0.055	0.198 ± 0.11	127.9 ± 38

The fish were fed twice a day at 9:00 a.m. and 2:00 p.m. For the morning feeding, 50 g of pellets were provided per tank and, in the afternoon, the fish were fed *ad libitum* to ensure that the animals were fully sated. Thirty minutes after the afternoon feeding, the remaining pellets were netted and removed from the tanks, and the pellets were counted to calculate the exact amount of ingested feed.

Experimental Diets

In order to meet the nutritional requirements of European seabass and ensure good performance of the fish, the different feed compositions were prepared according to the current recommendations for this species [17,29,30]. All experimental diets, including the control, were manufactured by SPAROS LDA (Olhão, Portugal) using the same size and extrusion parameters, to minimize technological differences among the feed trials.

Based on the formulation of commercial seabass feed, four different alternative, isonitrogenous feed concepts were produced, in addition to a control diet (Table 2). All diets were extruded floating pellets with a size of 6 mm. In addition, a typical commercial diet for seabass was mimicked for the control diet, based on analysis of commercial feed labels from different commercial suppliers and further contacts with feed formulators in the industry. The no processed animal protein (NOPAP) and NOPAP$^+$ diets included insect meal, fermentation biomass products, and vegetable protein concentrates; the processed animal protein (PAP) diet included poultry meal, feather meal hydrolysate, porcine blood meal, insect meal, and fermentation biomass products; and the PAP$^-$ included poultry meal, feather meal hydrolysate, porcine blood meal, and fishmeal. All alternative formulations contained hydrolysates from aquaculture by-products and macroalgae. The NOPAP$^+$ diet was formulated to maximize the performance of the fish and, therefore, supplemented with krill meal according to Torrecillas et al., (2021) [32]. All four alternative diets had significantly reduced levels of fish oil and rapeseed oil compared to the control; salmon oil and algae oil were used in this replacement. The NOPAP$^+$ diet was used as a positive control for fish growth performance, due to a higher proportion of high-quality FM, and the PAP$^-$ diet was used as a negative control, due to higher proportions of plant-derived proteins, to identify the thresholds of growth performance in *D. labrax*. Once the experimental diets were produced, they were directly delivered from Portugal to the experimental RAS facility at the AWI. Before and during the trials, the feed was stored at 4 °C to ensure continuous quality of the diets throughout the feeding experiment. The feed arrived, and the acclimatization period of the fish in the new tanks began; the feed was stored for two weeks before fish feeding started.

Table 2. Diet formulation of the experimental diets (% dry weight).

Ingredients, %	Control	NO PAP	PAP	NO PAP$^+$	PAP$^-$
Fishmeal Super Prime	10.00			15.00	
Fishmeal 60 (by-products)	5.00				
Krill meal				5.00	
Fish protein hydrolysate	3.00				
FPH-trout-head	0.50		0.50	0.50	0.50
FPH-trout-tf	0.50		0.50	0.50	0.50
FPH-turbot-head	0.25		0.25	0.25	0.25
FPH-turbot-tf	0.75		0.75	0.75	0.75

FPH-salmon-head		0.50	0.50	0.50	0.50
FPH-salmon-tf		0.50	0.50	0.50	0.50
Feather meal hydrolysate			5.00		10.00
Porcine blood meal			2.25		5.00
Poultry meal	10.00		14.00		20.00
Insect meal (*Hermetia illucens*)		15.00	10.00	10.00	
Fermentation biomass (*Corynebacterium glutamicum*)		5.00	5.00	2.50	
Fermentation biomass (*Methylococcus capsulatus*)		15.00	10.00	10.00	
Soy protein concentrate	4.40				
Pea protein concentrate		2.50		3.50	
Wheat gluten	6.00	1.50		1.50	
Corn gluten meal	6.00	1.50		1.50	
Soybean meal 48	15.00				
Sunflower meal 40		9.10		6.00	13.60
Wheat meal	11.40	5.70	5.70	5.70	5.70
Whole peas	4.00	11.13	16.36	9.14	14.44
Pea starch (raw)	4.00	4.00	4.00	4.00	4.00
Vit. and Min. Premix— WITH I and Se	1.00				
Vit. and Min. Premix—NO I and Se		1.00	1.00	1.00	1.00
GAIN Macroalgae SHP		2.50	2.50	2.50	2.50
GAIN Macroalgae SHP Se- rich		0.10	0.10	0.10	0.10
GAIN Microalgae WUR Se- rich		0.20	0.20	0.20	0.20
Vitamin E50	0.03	0.03	0.03	0.03	0.03
Betaine HCl	0.10	0.10	0.10	0.10	0.10
Antioxidant	0.25	0.25	0.25	0.25	0.25
Sodium propionate	0.10	0.10	0.10	0.10	0.10
Monoammonium phosphate	1.30	2.65	1.85	1.45	1.60
L-Histidine		0.10			
L-Tryptophan	0.10	0.10	0.10		0.25
DL-Methionine	0.20	0.40	0.30	0.10	0.30
L-Taurine		0.17	0.09	0.01	0.06

Yttrium oxide	0.02	0.02	0.02	0.02	0.02
Lecithin		0.25	0.25		0.25
Fish oil	5.40	2.70	2.70	2.70	2.70
Salmon oil		9.00	9.00	13.60	9.00
Algae oil		1.00	1.00	1.00	1.00
Rapeseed oil	12.70	5.90	5.10		4.80
total	100.00	100.00	100.00	100.00	100.00

FPH: fish protein hydrolysates; I: iodine; Se: selenium; SHP: Salten Havbrukspark AS, partner that produced macroalgae; VPC: vegetable protein concentrate; YM: yeast meal; WU: Wageningen University, partner that produced microalgae.

Measurements and Sampling

At the beginning of the experiment and every four weeks, the fish were anesthetized in a bucket with 500 mg/L^{-1} tricaine methanesulphonate (MS-222; Sigma Aldrich, Taufkirchen, Germany) for three minutes. The fish were individually weighed to 0.2 g precision, and measured in length to 0.5 cm precision and identified by their tags to measure the growth performance. At the beginning of the experiment (after the acclimatization period), 15 fish were anesthetized and sacrificed to obtain baseline data for the quality of their fillet, blood, and organs. After 83 days, at the end of the entire trial, five fish per tank were sampled individually for the sampling of their tissues, organs, and blood. Additionally, five fish per tank were pooled at the end of the trial to analyze the mineral data, and, furthermore, again, five fish per tank were pooled to analyze the proximate composition. After the animals have been anesthetized, blood was taken with a heparinized syringe, and half of the blood sample was transferred to an EDTA tube with a glycolysis inhibitor (potassium). The tubes were centrifuged at 2000× *g* at 7 °C for 10 minutes. The plasma was pipetted into an Eppendorf tube and stored at −20 °C for further analysis. Afterwards, the fish were decapitated and tissues (liver, head kidney, and fillet without skin) were sampled rapidly and put on ice. The liver was weighted with 0.1 mg precision to determine the hepatosomatic index (HSI). All tissues were shock frozen in liquid nitrogen and stored at −80 °C until further analysis. For digestibility analysis, the feces were sampled by a collection device per one tank (meaning three tanks per diet and 15 tanks in total). The collection device was installed under each tank and was emptied before the feeding in the morning and collected after a 4 h digestion period. The feces were centrifuged at 4° C and 3000× *g* for 5 min, and the supernatant was removed and frozen at −80 °C until further analysis. For the fillet analysis, five fish per tank were stunned with a head blow and then killed with a gill cut. All fillets were removed and weighted for the total yield and frozen at −20 ° C for further analysis.

Homogenization of Diets, Whole Body, and Feces

The diets were homogenized in a knife grinder (5000 rpm, 30 s, Grindomix GM 200, Retsch, Germany) two times to achieve homogenized materials and deep-frozen for further analysis. The collected feces were pooled per tank (n = 15) and freeze-dried for 24 h. Afterwards, the freeze-dried samples were homogenized in a knife grinder (5000 rpm, 30 s, Grindomix GM 200, Retsch, Germany) and stored at −20 °C for further analysis. Whole fish samples were pooled from three individuals. The fish were minced frozen in a commercial meat grinder, refrozen at −20 °C, and then freeze-dried for 48 h.

Chemical Analysis

Moisture, Ash, and Energy Analysis

The moisture content and ash of the experimental diets, feces, and whole-body fish was determined after AOAC (1980). The moisture content of the feeds was determined by drying the samples at 105 °C for 24 h. The moisture content of the feces and whole body was determined by freeze-drying for 24 h for the feces and 48 h for the whole-body samples. Total ash content was determined by combustion of the samples in a muffle oven at 550 °C for 6 h.

Gross energy was measured in an adiabatic bomb calorimeter (Model 6100, Parr Instrument, Frankfurt am Main, Germany).

Crude Fat and Crude Protein Analysis

The crude fat and crude protein analysis of the feed, as well as the whole-body samples, were conducted in an external lab (Labor IBEN GmbH, Bremerhaven, Germany). The crude fat was analyzed after Weibull/Stoldt (ASU L 06.00-6 2014-08* (Modification: Extraction with Soxtherm) and the crude protein with N × 6.25 (ASU L 06.00-7 2018-06*). For all other samples (feed, carcass, and fillet), the measured total nitrogen was converted to equivalent crude protein (%) using a conversion factor of
6.25. Crude lipid was determined after Weibull-Stoldt.

Mineral Analysis

The mineral analyses of diets, feces, and whole-body composition were conducted in duplicates. For the analysis of the mineral content, 0.2 g of freeze-dried and homogenized samples of the experimental diets, feces, and whole body were digested in 3 mL nitric acid HNO_3 (65%, trace grade) in a microwave oven (CEM MARS5, Kamp-Lintfort, Germany) according to DIN EN 13805 (2014). After digestion, the samples were diluted with milli-q water to 50 mL. Calcium, potassium, magnesium, phosphorus, arsenic, copper, iron, manganese, yttrium, and zinc concentrations were analyzed in an ICP-OES (iCAP7400, Fisher Scientific, Schwerte, Germany). Fish muscle (ERM—BB422, EU) was used as reference.

Blood Parameters

All blood parameters, except for the lysozyme activity, were analyzed in an external lab in Bremerhaven, Germany (Labor Dr. Schumacher MVZ GmbH, Bremerhaven, Germany). The following parameters were measured: potassium, sodium, calcium, lactate dehydrogenase (LDH), total proteins, and glucose. LDH, glucose, and total protein were analyzed with a photometer at 700 nm. The total protein content was analyzed after the biuret method and glucose after the hexokinase method; for LDH, lactate was transformed into pyruvate (IFCC method). Potassium, natrium, and calcium were analyzed with an ion-selective electrode method, in which a selective membrane measures the ions of each parameter.

Lysozyme Activity

The lysozyme activity was performed to the protocol of Milla [33]. The phosphate buffer consisted of 0.05 mol/L NaH_2PO_4 + 0.05 mol/L Na_2HPO_4 and was modified with 85% H_3PO_4 to a pH of 6.2. A total of 30 mg of *Micrococcus luteus* (0.6 mg/mL, SIGMA M3770) was mixed with 50 mL buffer on a daily basis, while 20 mg lysozyme from egg whites (Lot SLCC4285, 40382 Units/mg, Sigma L6876) was mixed with 20 mL of buffer weekly. The lysozyme–buffer solution was diluted to obtain 1000 U/mL. Therefore, 248 µL lysozyme solution was buffered in 9.752 mL buffer. A standard curve was created that ranged from 100 U/mL to 900 U/mL, as well as two blank samples (one with the bacterium and one with the buffer). For the samples, 10 µL and 5 µL of plasma with 10 µL and 15 µL of buffer were pipetted into the wells to obtain a volume of 20 µL per well. Immediately before starting the measurement, *M. luteus* was added at 130 µL to the standard curve and all sample preparations. For the measurement, 96-well plates (Brandplate 781660) with a clear flat bottom were used and measured in a Berthold Tristar LB941. The measurement was performed at 450 nm every minute over a period of 10 min. The plate was shaken for 5 s before the first measurement. For each measurement, the standard curve, samples, and blanks were measured in triplicate. Between measurements, the solutions were stored as much as possible in the refrigerator at 4 °C and the samples in the freezer at −20 °C.

Fillet Analysis

Sensory Analysis

The sensory analysis of the fillet was conducted in an external lab (Labor IBEN GmbH, Bremerhaven, Germany). The fillets were tested for their consistency before and after cooking, smell, taste, color, juice and grease separation, and protein precipitation with an ASU I 0.90-6 2015- 06 standard Norm method. The sensory analysis was carried out in the sensory room of the Labor IBEN by at least two test persons. The testers describe the test samples individually or jointly using descriptive expressions of their choice or based on predefined lists (for fresh fish, for example, according to the Karlsruher Scheme). The samples were cooked, and the temperature of the test samples was the same for each test person at the time of presentation.

The testing room was clean and odor-free at all times. Lighting was uniform, glare-free, and as close to the daylight spectrum as possible. The appearance, odor, taste, and consistency of the food were assessed.

Calculation and Formulas

The growth parameters were based on body weight (BW) and body length (BL) and was calculated as follows:

$$Weight\ gain\ (WG)\ (\text{g}) = BW\ final\ (\text{g}) - BW\ initial\ (\text{g})$$

$$Relative\ growth\ rate\ (RGR, \%\ ) = 100\ (e \frac{\ln(BW\ final\ (\text{g}) - \ln(BW\ initial\ (\text{g})))}{feeding\ days} - 1)$$

$$Condition\ factor\ (CF\ \%) = 100 \times \frac{BW\ final\ (\text{g})}{BL\ final^3\ (\text{cm})}$$

$$Hepatosomatic\ Index\ (HSI\ \%) = 100 \times \frac{liver\ weight\ (\text{g})}{BW\ final\ (\text{g})}$$

$$Viscerosomatic\ Index\ (VSI\ \%) = 100 \times \frac{viscera\ weight\ (\text{g})}{body\ weight\ (\text{g})}$$

The feed performance parameters, daily feed intake (DFI), and feed conversion ratio (FCR) were based on the feed intake (FI) in g of the offered amount of feed and the uneaten feed. Total FI and WG for FCR were corrected for the lost biomass through mortalities and sampling.

$$Total\ feed\ intake\ (F)\ total\ (\text{g}) = (Feed\ offered) - (Feed\ uneaten)$$

$$Daily\ feed\ intake\ (DFI, \%\ BW == 100 \times \frac{FI\ total\ (\text{g})}{(Feeding\ days \times \frac{(BW\ final\ + BW\ initial)}{2})}$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{FI\ total\ (\text{g})}{WG\ (\text{g})}$$

$$Protein\ efficiency\ ratio\ (PER) = \frac{weight\ gain\ (\text{g})}{crude\ protein\ intake\ (\text{g})}$$

The apparent digestibility (ADC) of the dietary nutrients and the apparent availability (AA) of minerals were based on the amount of the inert yttrium marker in the diet and feces and the respective nutrient or element in feces and diets.

$$ADC\,drymatter(\%) = 100 - (100 \times \frac{yttrium_{diet}}{yttrium_{faeces}})$$

$$ADC\,nutrient(\%) = 100 - (100 \times (\frac{yttrium_{diet}}{yttrium_{faeces}} \times \frac{nutrient_{faeces}}{nutrient_{diet}}))$$

$$AA\,(\%) = 100 - (100 \times (\frac{yttrium_{diet}}{yttrium_{faeces}} \times \frac{element_{faeces}}{element_{diet}}))$$

Statistical Analysis

Statistical analysis was conducted with Sigma Plot (12.5, Systat Software, Erkrath, Germany). One-way analysis of variance (ANOVA) was used to determine significant differences between the treatments. Whenever there were statistically significant differences, an all pairwise multiple comparison procedure was performed using the Holm–Sidak method overall significance level $p < 0.05$ to find the difference within the treatments. Values are given as means ± standard deviations. The sensory analysis was an ANOVA on ranks performed to see the statistical difference ($p < 0.05$). If statistical differences were detected, all pairwise multiple comparison procedure (Tukey test) was performed to detect differences over the significance level between the treatments.

Results

Feed

The proximate composition of feed can be seen in Table 3. The moisture was significantly higher in the PAP^- and $NOPAP^+$ diets. The energy was significantly lower in the PAP diet and dry matter was significantly lower in the PAP and PAP^- diet compared to the control diet (Table 3).

Table 3. Chemical composition of the experimental diets.

Feed	Control	PAP	NOPAP	PAP^-	$NOPAP^+$	*p* Value
Ash (%)	7.98^{b}	8.10 ^{ab}	8.23 ^{a}	8.06 ^{b}	8.04 ^{b}	0.039
Moisture (%)	8.10 ^{c}	8.88 ^{d}	8.88 ^{d}	10.19 ^{b}	11.30 ^{a}	<0.001
Gross energy (MJ kg^{-1})	23.16 ^{a}	21.58^{b}	22.88 ^{a}	23.08 ^{a}	23.14 ^{a}	<0.001
Crude protein (%)	37.9	39.0	38.5	35.8	41.0	0.406
Crude fat (%)	22.2	22.2	21.8	21.2	21.5	0.406
Apparent digestibility coefficient						
Dry matter (%)	79.18 ± 0.93 ^{ac}	77.59 ± 1.13 ^{bce}	76.24 ± 1.88 ^{acd}	76.14 ± 2.56 ^{de}	80.52 ± 1.01 ^{ab}	0.032
Gross energy (%)	74.95 ± 1.46	75.143 ± 1.17	73.60 ± 1.46	73.43 ± 1.53	70.48 ± 6.56	0.440

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), *p* values from one-way ANOVA.

The mineral composition of the feed can be seen in Table 4. All diets had an yttrium marker inside their formulation to later be able to analyze the apparent digestibility (ADC) of the different nutrients/minerals. The minerals were tested without replicates, as prior analysis determined very small variations within the mineral analysis of feed.

Table 4. Mineral composition of the experimental diets.

Feed	Control	PAP	NOPAP	PAP$^-$	NOPAP$^+$
Calcium (Ca; g/kg^{-1})	16.68	18.12	17.75	16.70	17.07
Potassium (K; g/kg^{-1})	6.72	6.43	6.51	5.41	6.44
Magnesium (Mg; g/kg^{-1})	1.82	1.71	1.77	1.57	1.67
Sodium (Na; g/kg^{-1})	4.04	3.73	3.85	2.31	4.47
Phosphorus (P; g/kg^{-1})	13.27	13.42	13.47	13.58	13.02
Ca/P ratio	1.26	1.35	1.32	1.23	1.31
Copper (Cu; mg/kg^{-1})	17.36	17.50	18.86	18.87	17.42
Iron (Fe; mg/kg^{-1})	346.04	198.41	180.04	259.99	162.49
Manganese (Mn; mg/kg^{-1})	34.73	32.38	34.39	37.78	25.51
Zinc (Zn; mg/kg^{-1})	173.97	161.39	171.89	181.72	179.55

Growth and Feed Performance

There were no mortalities during the feeding experiment. The fish fed the alternative diets showed no significant differences in total feed intake, FCR, or condition factor (Table 5).

Table 5. Performance parameters of the seabass fed five experimental diets (n = 45).

Feed	Control	PAP	NOPAP	PAP$^-$	NOPAP$^+$	*p* Value
Total feed intake (g)	261.92 ± 16.11	254.55 ± 5.76	247.27 ± 10.87	242.97 ± 8.22	258.85± 4.23	0.349
FCR	1.74 ± 0.04	1.96 ±0.16	1.67 ± 0.07	1.92 ± 0.17	1.79 ± 0.14	0.213
CF	1.20 ± 0.03	1.19 ± 0.02	1.19 ±0.03	1.19 ±0.01	1.21 ±0.02	0.817

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), p values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP$^-$: more plant-based materials (used as negative control); NOPAP$^+$: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

All fish had an initial weight of 320.8 ± 72.4 g. The weight gain at the end of the 83-day trial was significantly higher in control fed fish compared to the PAP and NOPAP treatments, as well as between PAP$^-$ and PAP fed fish (*p* value: 0.035).

The health indicator hepatosomatic index (HSI) showed no significant difference between the treatments, but the viscerosomatic index (VSI) differs significantly between the PAP$^-$ fed fish and the NOPAP$^+$ and control fed fish (*p* value: 0.009) (Table 6). The relative growth rate was significantly higher in control fed fish compared to all alternative diets: PAP$^-$, NOPAP, PAP and NOPAP$^+$ (*p* value: 0.009).

Table 6. Growth and health performance parameters of the seabass fed five experimental diets (n = 45).

Feed	Control	PAP	NOPAP	PAP$^-$	NOPAP$^+$	*p* Value
Weight gain (g)	159.90 ± 127.9 ^{a}	148.20 ± 129.2 ^{a}	144.00 ± 118.6 ^{bc}	138.55 ± 116.5 ^{bc}	144.50 ± 125.6 ^{ac}	0.035
HSI (%)	1.52 ± 0.37	1.41 ± 0.25	1.46 ± 0.26	1.47 ± 0.35	1.63 ± 0.32	0.438
VSI (%)	9.33 ± 1.52 ^{a}	8.97 ± 1.82 ^{a}	8.54 ± 1.42 ^{a}	8.30 ± 1.18 ^{b}	9.73 ± 1.77 ^{a}	0.009
BW final (g)	466.30 ± 105.22	475.68 ± 102.95	460.18 ± 88.28	459.33 ± 104.01	474.78 ± 101.47	0.847
RGR (%/day)	0.52 ± 0.13 ^{a}	0.48 ± 0.13 ^{b}	0.46 ± 0.09 ^{b}	0.45 ± 0.11 ^{b}	0.47 ± 0.09 ^{b}	0.009

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), p values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP$^-$: more plant-based materials (used as negative control); NOPAP$^+$: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

Whole-Body Composition

The whole-body analysis showed no significant differences between the crude fat, moisture, ash, or PER between all groups. However, the crude protein was significantly higher in the NOPAP fed diet compared to the PAP$^-$ and NOPAP$^+$ (p value: 0.022) fed fish (Table 7). The energy content of the NOPAP$^+$ fed fish was significantly higher compared to control, PAP, or PAP$^-$ fed fish (p value: 0.046).

Table 7. Whole-body composition parameters of seabass fed five experimental diets (n = 15).

Feed	Control	PAP	NOPAP	PAP$^-$	NOPAP$^+$	p Value
Moisture (%)	37.69 ± 2.33	37.45 ± 3.92	36.57 ± 2.83	38.61 ± 2.04	41.49 ± 7.05	0.296
Ash (%)	10.29 ± 1.10	10.42 ± 0.83	10.20 ± 0.64	10.45 ± 0.57	11.28 ± 3.71	0.845
Energy (MJ kg^{-1})	25.40 ± 1.37 ^{b}	25.11 ± 1.09 ^{b}	26.44 ± 1.92 ^{ab}	25.10 ± 1.31 ^{b}	29.90 ± 5.95 ^{a}	0.046
Crude fat (mg/kg)	16.47 ± 0.72	15.6 ± 0.7	16.37 ± 1.10	15.87 ± 2.20	15.45 ± 0.35	0.841
Crude protein (mg/kg)	18.03 ± 0.15 ^{a}	18.03 ± 0.15 ^{a}	18.57 ± 0.21 ^{b}	17.83 ± 0.40 ^{a}	17.75 ± 0.07 ^{a}	0.022
PER	1.56 ± 0.05	1.35 ± 0.11	1.56 ± 0.08	1.48 ± 0.11	1.36 ± 0.12	0.057

Values are expressed as means ± SD, values with different letters within the same line are significantly different (p < 0.05), p values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP$^-$: more plant-based materials (used as negative control); NOPAP$^+$: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

Mineral Analysis

The mineral content of the whole body showed no significant differences for the cultured seabass in any of the analyzed mineral and trace elements (Table 8).

Table 8. Analyzed mineral concentration on a wet weight basis in the whole body of seabass fed with five experimental diets for 83 days (n = 15).

Feed	Control	PAP	NOPAP	PAP$^-$	NOPAP$^+$	p-Value
As (mg/kg)	0.0004 ± 0.00	0.0001 ± 0.00	0.0003± 0.00	0.0003 ± 0.00	0.0002 ± 0.00	0.075
Ca (g/kg)	29.67 ± 2.61	28.67 ± 3.36	30.42 ± 0.45	33.61 ± 5.95	26.45 ± 4.14	0.287
Cu (mg/kg)	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.00	0.002 ± 0.001	0.003 ± 0.000	0.124
Fe (mg/kg)	29.47 ± 3.69	30.59 ± 2.37	28.82 ± 7.44	28.96 ± 4.02	23.55 ± 6.33	0.522
K (g/kg)	9.26 ± 0.65	9.23 ± 0.25	9.00 ± 0.31	9.12 ± 0.53	9.26 ± 0. 15	0.924
Mg (g/kg)	1.06 ± 0.07	1.08 ± 0.03	1.04 ± 0.04	1.08 ± 0.11	1.03 ± 0.05	0.825
Mn (mg/kg)	6.42 ± 0.85	6.01 ± 0.79	5.71 ± 0.51	6.60 ± 1.30	5.12 ± 0.93	0.349
Na (g/kg)	3.12 ± 0.29	3.05 ± 0.13	2.99 ± 0.15	3.21 ± 0.23	3.03 ± 0.01	0.646
P (g/kg)	18.85 ± 1.58	18.48 ± 1.58	18.34 ± 1.09	20.55 ± 3.18	17.25 ± 1.95	0.422
Zn (mg/kg)	37.43 ± 0.83	36.54 ± 1.80	37.75 ± 2.89	37.84 ± 2.54	34.15 ± 2.57	0.302

Values are expressed as means ± SD, values with different letters within the same line are significantly different (p < 0.05), p values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP$^-$: more plant-based materials (used as negative control); NOPAP$^+$: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

The apparent availability of sodium was significantly lower in PAP^{-} fed fish than in all other fed diets (*p* value: <0.001). Iron availability was significantly lower in the NOPAP^{+} and PAP fed fish compared to control and PAP^{-} (*p* value: 0.021) fed fish. However, the PAP^{-} fed fish showed significantly higher zinc availability compared to control, NOPAP, and NOPAP^{+} (*p* value: 0.043) fed fish (Table 9).

Table 9. Apparent availability of minerals and trace elements on a wet weight basis in the whole body of seabass fed with five experimental diets for 83 days (n = 15).

Apparent Availability (%)	Control	PAP	NOPAP	PAP^{-}	NOPAP^{+}	*p* Value
Calcium (Ca)	21.33 ± 8.3	27.47 ± 11.92	34.22 ± 14.58	26.60 ± 12.90	14.54 ± 7.20	0.346
Potassium (K)	87.79 ±1.33	88.19 ± 0.29	88.04 ± 0.91	85.96 ± 1.83	87.82 ± 0.59	0.180
Magnesium (Mg)	−130.35 ± 0.81	−145.74 ± 29.20	−120.79 ± 22.04	−164.99 ± 32.26	−149.89 ± 2.85	0.193
Sodium (Na)	−298.73 ± 46.47 ^{a}	−295.86 ± 13.90 ^{a}	−276.14 ± 18.36 ^{a}	−545.01 ± 92.41 ^{b}	−229.73 ± 19.68 ^{a}	<0.001
Phosphorus (P)	55.59 ± 1.70	60.35 ± 8.20	60.62 ± 7.25	66.40 ± 7.70	59.47 ± 6.08	0.438
Copper (Cu)	67.26 ± 4.10	63.22 ± 5.75	67.03 ± 5.18	69.12 ± 7.85	65.17 ± 5.40	0.770
Iron (Fe)	23.82 ± 22.54 ^{a}	−2.84 ± 5.56 ^{bc}	7.75 ± 6.47 ^{ac}	25.66 ± 6.35 ^{a}	−5.07 ± 5.79 ^{bc}	0.021
Manganese (Mn)	31.59 ± 12.67	37.67 ± 19.18	43.48 ± 15.18	55.45 ± 10.1	23.29 ± 6.01	0.112
Zinc (Zn)	19.99 ± 3.24 ^{a}	31.45 ± 11.75 ^{ab}	26.40 ± 11.24 ^{a}	46.11 ± 9.78 ^{b}	21.22 ± 9.16 ^{a}	0.043

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), *p* values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP^{-}: more plant-based materials (used as negative control); NOPAP^{+}: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

Welfare Parameters

The plasma analysis showed no significant differences in glucose, lysozyme, and LDH activity. The total protein content in plasma was significantly lower between the NOPAP^{+} fed fish and the control, NOPAP, and PAP- fed fish, as well as between PAP^{-} and control fed fish (*p* value: 0.001) (Table 10).

Table 10. Main blood parameters of seabass fed with experimental diets for 83 days (n = 15).

Feed	Control	PAP	NOPAP	PAP^{-}	NOPAP^{+}	*p* Value
Lactatdehy-drogenase (U/L)	46.44 ± 32.49	51.84 ± 32.52	43.88 ± 33.51	57.58 ± 28.53	41.76 ± 27.43	0.226
Glucose (mmol/L)	91.36 ± 26.60	95.72 ± 24.06	87.67 ± 17.69	85.96 ± 23.29	94.24 ± 29.36	0.384
Total protein (g/L)	41.68 ± 4.08 ^{a}	40.07 ± 4.13 ^{ac}	40.07 ± 3.36 ^{ac}	38.68 ± 3.59 ^{bc}	36.76 ± 3.44 ^{b}	0.001
Lysozyme (U/mL)	290.33 ± 34.46	270.67 ± 49.53	275.96 ± 44.33	276.17 ± 77.07	294.77 ± 70.16	0.169

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), *p* values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP^{-}: more plant-based materials (used as negative control); NOPAP^{+}: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

The fatty acids palmitinacid, linoleicacid, linolenic acid, and DHA showed no significant differences between the fish fed different diets. However, oleic acid was significantly lower in the fish fed the PAP diet compared to the control and PAP^{-} fed fish (*p* value: 0.016) (Table 11).

Table 11. Main fatty acids of seabass fed with five experimental diets for 83 days (n = 15).

Feed/Fatty Acid (mg/g)	Control	PAP	NOPAP	PAP^{-}	*p* Value
Palmitinacid (16:0)	27.73 ± 13.67	25.29 ± 20.65	29.22 ± 24.86	27.90 ± 11.8	0.950
Oleic acid (18:1n–9)	46.86 ± 28.64 ^{a}	22.84 ± 10.17 ^{bc}	36.47 ± 28.64 ^{ac}	48.01 ± 18.36 ^{a}	0.016
Linoleicacid (18:2)	19.30 ± 10.69	16.79 ± 14.92	18.89 ± 11.48	19.80 ± 8.46	0.899
Linolenic acid(18:3)	4.97 ± 3.69	4.36 ± 4.54	5.53 ± 3.84	5.41 ± 2.84	0.830
EPA (20:5)	7.12 ± 2.55	5.53 ± 1.98	6.25 ± 2.19	5.51 ± 1.43	0.123
DHA (22:6)	8.72 ± 3.46	8.53 ± 2.08	10.07 ± 3.31	8.26 ± 1.76	0.306

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), *p* values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein rich diet. PAP^{-}: more plant-based materials (used as negative control); NOPAP^{+}: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

Sensory Analysis

A significant difference (*p* value: 0.001) could be seen in fillet weight between the fish fed the NOPAP^{+} diet compared to the control and all other alternative diets at the end of the trial (Table 12). Sensory analysis showed no significant differences in any of the tested parameters: consistency frozen fat separation, protein, juice separation, or taste (one-way ANOVA on ranks; Tukey test).

Table 12. Fillet yield (%) of seabass fed with five experimental diets for 83 days (n = 15).

Feed	Control	PAP	NOPAP	PAP^{-}	NOPAP^{+}	*p* Value
Filet weight (%)	37.89 ± 2.18 ^{b}	39.24 ± 2.87 ^{b}	37.60 ± 2.51 ^{b}	39.03 ± 2.02 ^{b}	41.20 ± 2.15 ^{a}	0.001

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), *p* values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP^{-}: more plant-based materials (used as negative control); NOPAP^{+}: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

Discussion

The present study examined whether the replacement of fishmeal (FM), fish oil (FO), and soy products in feed efficiency by alternative ingredients affect the performance of European seabass (*Dicentrarchus labrax*). Different formulation concepts were tested including ingredients, such as processed land-animal proteins, vegetable protein concentrates, insect meal, fermentation products, macroalgae, fish protein hydrolysates from aquaculture by-products, salmon oil, and algae oil. The literature shows that seabass is known to have good acceptability of plant and animal-derived protein replacements at moderate levels [17,30,33,34]. The present study attempts to test whole feed alternative formulation concepts, with the replacement of more than one ingredient at a time. In the following, the different performance aspects are considered to better interpret the general health and growth parameters, first from a physiological point of view and later from the perspective of the consumer and/or farmer. The distinction between aquaculture candidate and farmer is critical here, as the farmer has to consider the best aspects for the welfare of the fish as well, while, on the other side, being economically feasible. For the consumer, it is the sensory analysis, as well as food safety in terms of toxic elements and healthy fatty acids.

Growth and Feed Performance

The present study examined the effect on growth and feed performances, and no significant differences were found in total feed intake, FCR, CF, or final body weight for all different diets. In contrast, the relative growth rate was significantly higher in the control fed fish (0.52%/day) than in all other diets (0.45—0.48%/day). The same could be seen for the weight gain, which was significantly higher in the control and PAP fed fish compared to the other diets. The significant weight gain but insignificant final weights can be explained by the high variability in the initial weights. Health indicators, such as viscerosomatic index (VSI) (7–13%) and hepatosomatic index (HSI) (1.5—2.3%), are congruent with the recent literature [19]. The HSI (1.4—1.6%) showed no significant differences between the different treatments, indicating good health performance for all feeding concepts. The VSI, on the other hand, shows significantly lower values for the PAP fed fish (8.3 ± 1.18) compared to all other feed concepts (8.54–9.73). This result was expected as it is already described in the literature that more plant-based diets can lower health performances in carnivorous fish [13,15,16,17]. Comparing the different feed concepts and growth parameters, we see that the control and PAP fed fish had the best performance, closely followed by the NOPAP+ and NOPAP fed fish, and PAP fed fish with the lowest performance. Overall, these results indicate that the control diet can be exchanged for one of the more sustainable alternative diets (PAP or NOPAP) with no adverse effect on growth. This is in line with other studies investigating European seabass using less radical alternative formulations [19,30,35].

Whole-Body Composition

The whole-body composition did not show significant differences in moisture and ash between the control and alternative diets and is congruent with the recent literature [19,23,33]. Crude fat (here: 15.45– 16.47 mg/kg) is well within the normal range for European seabass (7– 21 mg/kg) and does not significantly differ between the different feed concepts [19,28,33,42]. Crude protein is significantly higher between the NOPAP fed fish (18.57 mg/kg) and the other feed concepts (17.75–18.03 mg/kg) but still well within the range of the literature (15–22 mg/kg) [19,28].

The protein efficiency ratio (PER) range of 1.35–1.56 is well within the range described by the literature and shows a good ratio within all experimental and control fed fish [36]. Higher lipid levels in the diet are already known to improve feed efficiency and PER [36]. Fish that obtained the highest PER in this study were fed the commercial and NOPAP diet, with lipid levels of 22.2% and 21.8%. The literature shows a decrease in PER in seabass with increasing dietary protein beyond the optimum level [36-38]. This can be observed in the PER of the PAP and NOPAP+ fed fish, which had crude fat levels of 39% and 41%. These findings are congruent with the literature that shows no body compositional decrease by plant- [34] and terrestrial-animal- derived protein replacements of up to 30% [19,28,39]. The conversion factor used in this study was an estimate for all diets. In an experimental design, such as the one described here, with feed concepts rather than individual ingredients, the exact conversion factor calculation is complex and not feasible for the farmers. That is why we determined the factor nutritionally, which gives a good overview of the respective concepts. Therefore, it was concluded that the applied alternative feed concepts do not negatively affect the biochemical composition of seabass.

Mineral Analysis

No significant differences in mineral concentrations were found between control and replacement feed concepts, which is congruent with the literature replacing fishmeal up to 33% [18,40,41]. Furthermore, low values of feed-derived toxic elements, such as arsenic, were found in all fish, independent of the feed concept. Arsenic is known to impair growth, especially in children, and the lower the value, the less toxicity can be assumed, leading to safer end products [22,30]. Since wild fish contain higher amounts of arsenic (1.030–1.230 mg/kg), fish fed with the experimental diets are safer for human consumption, an important positive aspect in cultured fish [42]. Interestingly, a significant decrease in arsenic in the PAP fed fish can be seen, which could be interpreted as a better option for human health than the control diet fed fish. This study indicated that iron and zinc (in the ranges of 23–30 and 34–37 mg/kg, respectively) were the main micro-mineral components, similar to that observed in other studies in cultured seabass [4]. Zinc is known to have significant healing, antioxidant, and immunostimulating properties in carnivorous fish, and a high availability, such as in the fish of this study, is wanted to ensure high health standards [43]. In general, copper, iron, and zinc are known to be nutritionally vital metals and can have positive effects on human health [18].

Plant-based feed ingredients are known to bind minerals and, thus, reduce the availability of phosphorus, iron, and zinc in fish [44]. In this study, no negative correlation between the alterative and more sustainable feed concepts (PAP and NOPAP) could be seen. However, it is known that calcium, phosphorus, and manganese are mainly accumulated in fish bones, heads, and gills, meaning a higher % of fishmeal leads to elevated levels of the listed minerals [18]. This study showed that the replacement of FM with aquaculture by-products in the presented feed concepts does not negatively affect the amount of the above minerals and is, thus, a good and more sustainable alternative than the commercial FM. In general, it can be said that the ingredients in feed can have two opposing effects: (1) supplying minerals and (2) supplying substances that reduce the absorption of minerals [45]. Therefore, the availability of nutrients can be explained as the total of these two effects. When the latter effect is larger, the availability is somewhere below 0%, resulting in negative values like in sodium and magnesium. Our study showed that higher proportions of bone materials, such as in the PAP$^-$ diet, lead to reduced availability of the total amounts of minerals [45]. Since the mineral concentrations in the whole body are similar in the fish from all diet concepts, it can be concluded that the mineral and trace element demand was sufficiently covered and that elevated excretion rates balanced the elevated mineral concentration in the experimental diets. Therefore, fish are a good source of essential minerals, and this study showed that the alternative diets tested here do not negatively affect the mineral composition and can replace up to 17% of FM [41].

Welfare Parameters

Among others, plasma metabolites, such as glucose and total protein, are considered as essential and sensitive indicators for the physiological changes due to stress and diseases [46]. In this study, plasma glucose (41.7–57.6 mmol/L), LDH (41.76–57.58 U/L), and lysozyme levels (270–294 U/mL) were not affected by alternative diet fed fish, congruent with the literature where up to 15% of plant material was replaced without adverse effect [17,23,24,33]. In contrast to that, a decrease in total protein content can be seen between control (41.68 g/L) and PAP$^-$ (38.68 g/L)/NOPAP$^+$ (36.76 g/L) fed fish. These values are congruent with the literature and may imply that seabass is either even more tolerant of substandard feed mixtures, especially PAP$^-$, or that the NOPAP$^+$ mixture performed below expectations and targets in terms of, e.g., bioavailability of nutrients [47]. The total protein content in the plasma can be seen as a health indicator, as an elevated level would indicate a poor immune system. The low values in the PAP$^-$ diet were expected and can be overlapped with the low VSI, another indicator for poor health. The welfare status is a very complex system, and many factors need to be considered to understand the picture entirely. For the NOPAP$^+$ diet, we can see a lower value on protein content in the plasma, but none of the other measured parameters indicate poor welfare. This concludes that welfare is not necessarily negatively affected in NOPAP$^+$ fed fish. The lower values in the total protein in the plasma are more likely explained by the fact that too much protein content in the feed leads to less protein efficiency, as can be proven by the low PER in NOPAP$^+$ fed fish. The sustainable alternative diets, PAP and NOPAP, induced no significant differences in the blood parameters and can, thus, be considered to provide a similar welfare status of the fish when exchanged for the less sustainable control diet.

When looking at the welfare status of fish, it is crucial to take highly unsaturated fatty acids (HUFAs) into account, which are mainly responsible for the metabolism of fish and generate growth and immune function [28,48]. Furthermore, especially in farmed animals, higher stress is given due to handling, densities, and transportation, which make the health status of fish even more essential, and amino acid need to be considered a critical welfare category. Values for all fatty acids were within the range of European seabass and showed no significant differences for any fatty acids, except the oleic acid, compared to the control fed fish vs. alternative diet fed fish [18,49]. Palmitic acid between 12 and 29 mg/g, oleic acid between 31 and 44 mg/g, linoleic acid between 7 and 35 mg/g, linolenic acid between 1 and 5 mg/g, EPA between 1.5 and 7 mg/g, and DHA between 4 and 13 mg/g were well within the range of the literature and do not indicate poor welfare status [18,49]. The most important fatty acids for human consumption are linolenic acid, EPA, and DHA, which are well-researched and function in important metabolic processes for human health and are well-expressed in fish from this study, supporting fact for safe and eco-friendly seafood [18,49,50]. In today's society, healthy food is critical, as more and more contaminants (whether vegetables or meat) are found, and consumers are confused in their purchasing decisions. Overall, the welfare parameters are not negatively affected when the control diet is replaced by one of the more sustainable feed concepts with processed animal (PAP) or plant proteins (NOPAP).

The exchange of commercial FM with more sustainable, recycled fish hydrolysates from aquaculture by- products additionally does not show any negative effect on the fish welfare. This supports the hypothesis that the alternative feed concepts presented here can replace the less sustainable commercial feeds.

Sensory Analysis

In the fish fed NOPAP+, the fillet yield was higher, with a mean value of 41.2%, probably because NOPAP+ was enriched with Super Prime fishmeal and krill meal, which are known to produce better growth characteristics [34]. Interestingly, the alternative diets, PAP (39%) and NOPAP (38%), indicate no significant differences to the control-diet-fed fish (38%), resulting in the conclusion that the alternative formulation concepts do not negatively affect the fillet yield.

Sensory attributes are the most crucial factor for consumers, and the results of this study indicate that the control diet can be replaced by more sustainable alternatives (PAP and NOPAP) without affecting sensory attributes. According to the literature, softness, juice separation, taste, and smell are the most relevant attributes for consumers [51]. In this study, those attributes were not affected by alternative-diet-fed fish, in accordance with the recent literature for seabass (*D. labrax*) [50,52], greater amberjack (*Seriola dumerili*) [40], and gilthead seabream (*Sparus aurata*) [53]. The health benefits of fish from recirculation systems are apparent. This study shows that the values for fatty acids and the low toxic elements could encourage consumers to buy more fish. In addition, this health advantage of fish products does not fall in favor of a negative correlation between taste or consistency. This can be seen as a success for the effort to develop alternative and more sustainable feed concepts without losing sensory attributes. From an ecological point of view, the introduction of sustainable feeds is essential, but the economic difference between feeds should also be considered.

The most crucial factor in selecting new feed is to calculate the costs right from the beginning to the farm. This study tested four different feed concepts that could potentially replace the commercial feed for seabass. However, the raw materials used here were selected on an experimental basis, making estimating costs impossible due to excessive imprecision. By-products from the circular economy and artificially enriched algae were utilized for the sustainable ingredients in our diets. These are currently only used on a scientific basis and not commercially so that no realistic cost assumptions can be calculated. For the future, it would, therefore, be essential to make the by-products (such as fish hydrolysates from aquacultures) as well as enriched algae for fish feed available for commercial use.

Conclusions

This study shows that alternative diets using emerging and novel plant and animal-derived protein sources (both terrestrial and aquaculture by-products) have comparable performance to current commercial seabass feeds while being more sustainable. The fish welfare and sensory quality, which seem to be the strongest drivers for consumer appreciation, were not negatively affected by more sustainable feed concepts in which FM, FO, and soy products were replaced by several alternative ingredients (NOPAP and PAP). Furthermore, the PAP and NOPAP diets additionally had these traditional ingredients replaced by agro-industry, fisheries, and aquaculture by-products, to get into the direction of circular economies. The usage of aquaculture by-products in comparison to commercial FM has far less transportation distances (as it is usually reused at the same site) and additionally recycles the resources at hand, which make the overall production more eco-friendly. Therefore, this study provides evidence that alternative diets can successfully replace commercial diets for European seabass. The transfer to more sustainable diets would positively affect the consumers' attitude towards farmed fish (better sustainability with the same welfare and sensory status) and the fish farmers' potential for further sustainable and circular-economy-driven industrial growth. The recommendation for future feed formulations for farmed European seabass would be to replace the FM completely with hydrolysates, exchange the SBM with more sustainable plants, such as wheat and enriched algae, and add agriculture by-products, such as feather meal, etc., for a better mineral composition and digestibility.

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Chapter V

Circadian rhythm in turbot (*Scophthalmus maximus*): Daily variation of blood metabolites in recirculating aquaculture systems

Manuscript in preparation; Journal to be decided

Circadian rhythm in turbot (*Scophthalmus maximus*): Daily variation of blood metabolites in recirculating aquaculture systems

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Metabolism, NMR spectroscopy, metabolomics, turbot, daily rhythm, stress physiology, circadian timing

Abstract

Aquaculture is an ever-growing food sector and offers the greatest potential for providing the world with sustainable protein sources. Animal welfare in aquaculture is therefore becoming increasingly important, and detailed knowledge of individual species is essential for further optimization. Every organism is controlled by an internal clock, the circadian rhythm, which is crucial for metabolic processes. The circadian rhythm is particularly influenced by abiotic factors such as light and temperature, which are usually manipulated in aquaculture operations to increase productivity. Therefore, understanding the circadian rhythm of aquaculture fish is critical to maximize productivity and optimizing growth and welfare. Choosing the right feeding time according to the natural metabolism of the animal is important for both physiological and economic reasons. Turbot is a highly valuable fish and high-priced species which is typically farmed in RAS. This study is the first to investigate the circadian rhythm of adult turbot in plasma. Blood samples were collected over a 24-hour period and plasma metabolite profiles were analyzed by ^{1}H-NMR spectroscopy. As a result, 46 metabolites were identified in the blood, eight of which appeared to shift throughout the day. We noted increased values around 3 pm for many metabolites measured, which were especially high in for isoleucine, leucine, valine, phenylalanine, lysine, and lactate. These metabolic peaks could be interpreted as either habituation to the usual feeding time or as natural peak levels in turbot in a 24-hour circle. This study provides some initial insight into the daily variation of metabolites in adult turbot, and future studies will need to support our trend that shows increased values in amino acids and sugars at 3 pm. Previous studies did already show a correlation between immune function, stress and feeding ratio, which needs to be elevated for turbot to optimize their farming practices. Implementing optimized feeding times (like at times with high sugars and low in stress metabolites) could thus lead to less stress in the animal, leading to better health and welfare. These optimizations could additionally lead to lower feed conversion ratio, giving the farm a higher profitability and sustainability without extra costs for the farmer.

Introduction

Fish is widely considered an important source of healthy food, and accurate information on best farming practices is required to optimize cultivation and ensure sustainable production (Ahmad et al., 2021; Bennett et al., 2021; Farmery et al., 2022; FAO, 2022). Moreover, since fish are rich in proteins, micronutrients (such as vitamins and minerals), lipids, and omega-3 unsaturated fatty acids, they contribute to fighting hunger and malnutrition in the world (Golden et al., 2021; Mohanty et al., 2019). Unfortunately, animal welfare concerns often negatively influence consumer perceptions (Ankamah- Yeboah et al., 2019; Funk et al., 2021, Maesano et al., 2020). Enhancing fish welfare is, therefore, essential to improve eco-intensification and increase the appeal of aquaculture products to consumers (Brijs et al., 2018).

The concept of animal welfare is a multilevel approach due to species dependency, high individual variability, and interrelated complex metabolic processes (Ashley, 2007; Carbonara et al., 2020; Martos-Sitcha et al., 2020; Toni et al., 2019; Zheng et al., 2021). There is not one single measure of welfare but serval and the most recognized ones are indicators such as immune response, stress level, and feeding behaviour (Ashley, 2007; Toni et al., 2019; Tort, 2011). All organisms, including teleost fish, have a circadian rhythm (CR) that controls these indicators and changes periodically depending on external factors such as light, temperature, or feeding times (Choi et al., 2020; Fortes- Silva et al., 2019; Frøland Steindal et al., 2019; Nikkhah, 2015; Sanchez-Vazquez et al., 2019). It consists of multiple molecular processes as well as several clock genes such as *per, clock, bmal,* and others (Pilorz et al., 2018; Sanchez- Vazquez et al., 2019). These clock genes synchronize all metabolic functions according to the environmental conditions to increase fitness (Prokkola et a., 2018). The CR is thus, responsible for numerous cellular processes, including stress and immune responses as well as appetite (Ceinos et al., 2019; Nikkhah, A., 2015; Pilorz et al., 2018; Prokkola et al., 2018: Zheng et al., 2021). Previous studies have shown that reduced feed intake in fish is a distinct behavioural response to stress and affects not only growth but also their welfare and body composition, and thus yield and profitability for the farm (Assan et al., 2021; Dawood et al., 2020; Lopez-Olmeda et al., 2009; Yang et al., 2018). The CR is especially important for intensive aquaculture farms, which manipulate abiotic factors to increase their efficiency (Choi et al., 2020). Therefore, an optimized feeding regime improves feed conversion without extra costs for the farmer, resulting in higher profitability. Moreover, avoiding feed waste through proper feeding minimizes the loss of expensive feed and the accumulation of nitrogenous waste in the water, which could be toxic to the fish (Assan et al., 2021).

In intensive fish farms, fish are exposed to many different stressors, such as high stocking densities, handling, transportation, or restricted and unfamiliar environments (Assan et al., 2021; Long et al., 2019; Martos-Sitcha et al., 2020). Recent studies have shown that handling fish at different times of the day can result in likely lower stress reactions (Figueiredo et al., 2020; Lopes et al., 2022; Montero et al., 2019). Stress is generally defined as a disruption of physiological or biological mechanisms caused by internal and external factors commonly referred to as stressors (Barton, 2002; Hernández-Pérez et al., 2019; Ramsay et al., 2009; Wendelaar Bonga, 1997). Many indicators of stress exist, but cortisol is one of the best established and most frequently used in circadian rhythm studies (Cowan et al., 2017; Prokkola et al., 2018; Tort, 2011; Valencia et al., 2022). However, these stressors are experienced very individually by each species, and extrapolation from one species to another is highly speculative, making it necessary to study each species separately (Hernández-Pérez et al., 2019; Lopes et al., 2022; Pilorz et al., 2018; Zheng et al., 2021). In most commercial fish farms, however, animals are fed according to fixed feeding schedules instead of adapting to the animals' metabolism in a species-specific manner (Callier et al., 2017; Pratiwy et al., 202). In addition to cortisol as a stress marker, glucose is of critical importance in aquaculture systems as it affects nutrient availability and oxygen consumption of animals (Prokkola et al., 2018). Moreover, glucose, along with other sugars, is the main energy supplier of organisms as it is converted to adenosine triphosphate (ATP) during gluconeogenesis (Cowan et al., 2017). This is important because the availability of energy in the organism helps the animal to better cope with stressful situations (Valenzuela et al., 2022).

Previous studies have shown a close relationship between immunological and stress responses and thus the circadian rhythm in fish (Montero et al., 2019; Schleiermann et al., 2013; Valenzuela et al., 2022). Stress suppresses immune responses when fish are exposed to stressors such as feed intake, handling, or care/transport practices, and consequently increases the risk of disease outbreaks (Hernández-Pérez et al., 2019; Sakai et al., 2021; Skouras et al., 2003; Song et al., 2021). A good immune parameter is lysozyme. Levels in the blood are directly coupled to liver concentration (the most important lysozyme-producing organ) and show decreased levels during periods of high stress (Valenzuela et al., 2022). This has particular implications for fish health, and a better understanding could reduce the risk of disease outbreaks in aquaculture operations.

In general, these periodic shifts in metabolism affect hormone production and consequently stress levels, energy availability, food intake and utilization (Cowan et al., 2017). Therefore, it is critical to evaluate the processes occurring over 24-hours in order to adjust the feed timing (during stressed and non-stressed times of the day) to match the metabolism of the fish. Knowledge of the metabolism could help to improve the productivity of the farm by optimizing the feeding time of the animals, reducing their stress and consequently increasing the welfare and profitability of the farm.

To better understand the underlying processes in an organism, the use of metabolomics can help and allows the qualification of low molecular weight (< 1000 DA) metabolites (Cappello et al., 2019; Lin et al., 2006). Metabolomics is the study of endogenous metabolite profiles in biological samples (Macias et al., 2019; Samuelson et al., 2006). In fish, as in all other living organisms, there are many different types of molecules, such as lipids, water, proteins, carbohydrates, vitamins, amino acids, and other metabolomic compounds involved in metabolic processes (Hatzakis E. 2019). Metabolomics reflects changes in the physiological process and is therefore a powerful tool that provides information about the overall metabolic state of an organism (Samuelson et al., 2006). The presence of specific metabolites can help identify underlying processes and thus provide information about the animal's welfare, as well as identify times when the animal is exposed to increased levels of stress metabolites.

Nuclear magnetic resonance (NMR) spectroscopy is frequently used in metabolomics and is becoming increasingly important for aquatic organisms (Crook & Powers, 2020; Deborde et al., 2021; Roques et al., 2020; Tavares et al., 2022). Being a quantitative analytical tool, it is regarded as a nondestructive method and enables high reproducibility, quantitative metabolic profiling, rapid analyses, and cost-effectiveness (Cappello, 2020; Hatzakis, 2019; Samuelson et al., 2006). NMR spectroscopy provides unbiased assessment of metabolite data, making it an ideal tool for untargeted analyses, and it can be studied in cells, tissues, organs, plasma, or even whole organisms (Cappello, 2020; Crook & Powers, 2020; Lin et al., 2006;

Roques et al., 2020; Young & Alfaro, 2016). NMR-based metabolomics in plasma is of particular interest as it enables nonlethal as well as repeated sampling for continuous monitoring in fish (Tavares et al., 2022). That fact may be important to farmers, since it allows monitoring their fish's welfare without having to dispatch them.

This method can therefore help identify critical metabolic pathways and reveal changes in metabolites in turbot during the diurnal and nocturnal cycles. Turbot is a valuable marine fish species for aquaculture and has received remarkable attention in recent decades (Pyanov et al., 2021). Turbot are usually cultured in RAS facilities, and the growth and health of this species are well studied, but the areas of stress physiology and CR are still in the early stages of research (Fraga-Corral et al., 2022; Hoerterer et al., 2022). This study focuses on the identification of metabolites in the blood of turbot kept in a recirculating system. To our knowledge, no previous study has investigated the circadian rhythm in turbot to gain insight into its physiological processes. Such information is required in order to better understand the metabolism and make adjustments to aquaculture practices. Modifying feeding and handling times in intensive aquaculture operations to match species-specific circadian rhythms could contribute to higher growth and better feed conversion. Consequently, these changes could lead to optimized farm profitability and improved animal welfare without imposing additional costs on the farm.

Material and Methods

Experimental Setup

A total of 60 turbot (*Scophthalmus maximus*) kept at the ZAF (Centre for aquaculture research) from the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI) (Bremerhaven, Germany) were used for the experiments. The fish were acclimated in a recirculating aquaculture system (RAS) for two weeks before the start of the experiment. For acclimation, the fish were randomly and evenly distributed among twelve tanks. The average weight of the fishes was 551.94 g ± 102 g.

The experimental RAS consisted of 36 individual holding tanks, each given with a base area of 1 m² and a volume of 700 l. For the experiment, fifteen of the 36 tanks were actively used, twelve for the experiment itself and three other tanks as a reserve and for replacement fish. The water was treated with standard RAS purification devices such as a drum filter, a biofilter, a protein skimmer (with ozone), and a trickling filter. The condition (temperature, pH, salinity, oxygen) of the process water was constantly monitored using a SC 1000 Multiparameter Universal Controller (Hach Lange GmbH, Düsseldorf, Germany). Nutrient concentration (nitrite, nitrate, ammonium) was measured twice a week using the QuAAtro39 AutoAnalyzer (SEAL Analytical, Germany). There were no deviations in water values during the entire course of the experiment (Table 1).

Table 1: Mean values ± standard deviation of the water parameters ((temperature, pH, salinity, and oxygen: n = 83; ammonium, nitrite, and nitrate: n = 42)

Temperature (°C)	pH	Salinity	Oxygen (%)	Ammonium (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)
20.0 ± 0.9	7.62 ± 0.04	36.3 ± 0.1	95.2 ± 6.7	0.096 ± 0.055	0.198 ± 0.11	127.9 ± 38

Twice a day at 9 a.m. and 3 p.m. over a period of 6 month, the fish were fed with a commercial diet *ad libitum* in order to acclimate to those feeding times (see Hoerterer et al., 2022 for detailed information). Twenty-four hours before the start of the experiment, all experimental fish were starved (Jia et al., 2018).

Measurement and sampling

Every two hours during the experiment, a tank was randomly selected and all five fish were sampled. To be more precisely, at 7 a.m. the five fish of the first tank were netted and sampled, followed by the next tank at 9 a.m. This procedure was repeated until all the tanks were used, leading to a twenty-four hours cycle (after Hernández-Pérez et al., 2019 and Lopes et al., 2022). The fish were netted together and killed with a head strike followed by a neck cut.

The sampling procedure run as follows: First, the fish were weighed, measured in length, and placed directly on ice for blood collection. Second, blood was collected with a heparinized syringe in the caudal vein, and the Eppendorf tubes were directly centrifuged at 2,000 g and 7 °C for 10 minutes. Third, the supernatant (plasma) was taken and stored at -20 °C for further analysis. In order to ensure that metabolites were not further expressed, the entire process from netting to blood collection was kept within 5 minutes for all five fish together.

2.3. Lysozyme activity

The lysozyme activity in the plasma was photometrical measured according to the protocol of Milla et al., 2010. The phosphate buffer consisted of 0.05 mol/L NaH_2PO_4 + 0.05 mol/L Na_2HPO_4 and was modified with 85 % H_3PO_4 to a pH of 6.2 at room temperature. 30 mg of *Micrococcus luteus* (0.6 mg/mL, SIGMA M3770) were mixed with 50 mL buffer on a daily basis, while 20 mg lysozyme from egg whites (Lot SLCC4285, 40382 Units/mg, Sigma L6876) were mixed with 20 mL of buffer weekly. The lysozyme- buffer solution was diluted to get 1,000 U/mL. Therefore, 248 µL lysozyme solution was buffered in a 9.752 mL buffer.

A standard curve was created that ranged from 100 U/mL to 900 U/mL, as well as 2 blank samples (one with the bacterium and one with the buffer). For the samples, 10 µL and 5 µL of plasma with 10 µL and 15 µL of buffer were pipetted into the wells to obtain a volume of 20 µL per well. Immediately before starting the measurement, *M. luteus* was added with 130 µL to the standard curve and all sample preparations. For the measurement, 96 well plates (Brandplate 781660) with clear flat bottom were used and measured in a Berthold Tristar LB941.

The measurement was performed at 450 nm every minute for ten minutes. The plate was shaken for five seconds before the first measurement. The standard curve, samples, and blanks were measured in triplicate for each measurement. Between measurements, the solutions were stored as long as possible in the refrigerator at 4 °C and the samples in the freezer at -20 °C to stop the expression of further metabolic products as much as possible.

(Mechanical) analysis of glucose, lactate and cortisol

For glucose, lactate and cortisol the blood samples were divided in half and send to our partner in Rostock for further analysis. The other half was analysed in ^{1}H-NMR spectroscopy, see 2.5.

Glucose and Lactate were tested with test strips and cortisol with an ELISA Kit.

NMR Analysis

Plasma samples were prepared by methanol extraction prior to NMR analysis according to Nagana Gowda & Raftery, 2017.

600 µL of plasma were mixed with 1200 µL of HPLC methanol and shaken for 30 seconds. Samples were then incubated at -20 °C for 20 minutes and centrifuged at 13,000 g at 3°C for 30 minutes. Next, the supernatant was transferred to a new vial and dried overnight in a vacuum concentrator (SpeedVac, RVC 2-33 IR, Christ GmbH, Germany). The dried samples were mixed with 600 µL of D_2O (Sigma, Nr.775002) containing TSP standard (TSP; 0.05 wt%; Sigma Aldrich, St. Louis, USA) as an internal reference and centrifuged at 13,000 g at 7°C for five minutes. Finally, 500 µl of the supernatant was transferred to a 5 mm NMR tube. Spectra were measured in an ultra- shielded vertical 9.4 T NMR spectrometer (Avance III HD 400 WB, Bruker-BioSpin GmbH, Germany) using a 5 mm BBx Probe at a proton frequency of 400 MHz. All samples were analyzed by one dimensional ^{1}H–NMR spectroscopy using a cpmg protocol with water presaturation at 21 °C (cpmg1pr) within TOPSPIN 3.2 software (TopSpin 3.5pl, Bruker-BioSpin GmbH, Germany).

Spectra were processed and analyzed using Chenomix NMR Suite 8.0 software (Chenomix nmr suite 8.4 Professional). Before analysis, all spectra were manually corrected for phase, shim, and baseline and calibrated to TSP standard. Next, the metabolite peaks of the processed ranges were analyzed and assigned to their chemical compounds using the Chenomix database and literature as reference (Beckonert et al., 2007; Götze et al., 2020; Rebelein et al., 2018). Finally, the concentration of the assigned metabolites was determined by the Chenomix software based on the concentration of the internal standard (TSP set to 3.3 mM).

Statistical analysis

Blood samples from Rostock were analyzed using Sigma Plot (12.5, Systat Software, Germany). One-way analysis of variance (ANOVA) was performed to detect significant differences between time periods (p.value < 0.05). Results were expressed as means with standard deviations in the corresponding concentrations.

Statistics on metabolite profiles of NMR spectra was performed using the online platform MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/, after Xia et al., 2016). Metabolites were normalized by cubic root transformation and autoscaling to stabilize the variation between metabolites. Then, one-way ANOVA was performed with Fisher's LSD post hoc test at an adjusted p. value of 0.05. In addition, a significance analysis of metabolites (SAM) was performed with equal group variances and a false discovery rate (FDR) of 0.3. Significant differences between groups of altered metabolites were plotted using Sigma Plot (12.0, Systat Software, Inc.). Unsupervised principal component analysis (PCA) was performed and revealed no outliers (not shown).

Results

The fish had a weight of 551.94 g ± 102 g and a length of 30.79 cm ± 2.06 cm. No deaths occurred during the experiment (24 hours).

(Mechanical) analysis of lysozyme, cortisol, glucose and lactate

The results show no significant differences between glucose, lysozyme, and cortisol (Fig. 1 and 2, Annex: Table 2). Lactate levels changed over the 24h sampling time and were elevated at 3 am and 9 am (see Fig. 1). However, most lactate levels were below the threshold and could not be detected with the test stripes.

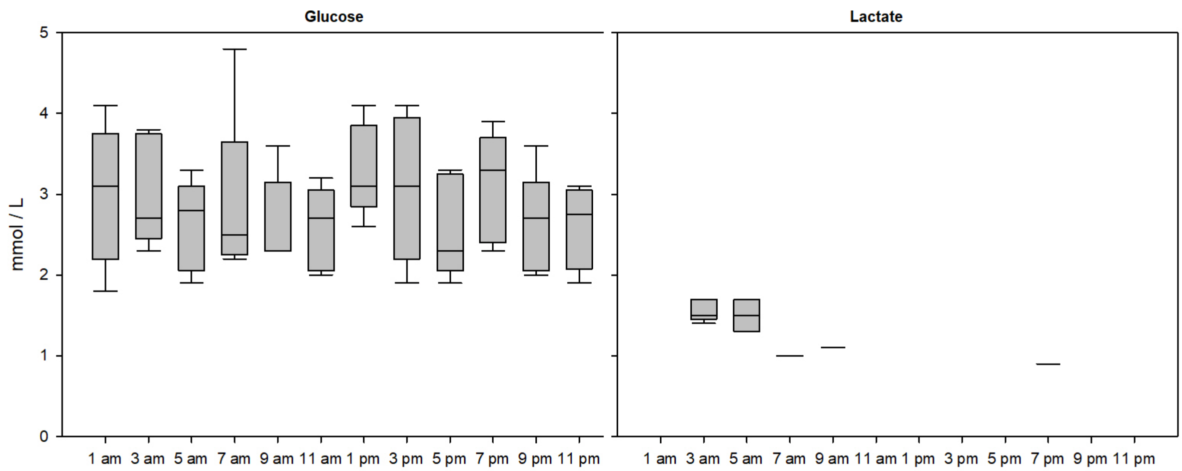

Figure 1 Metabolite levels in plasma of *Scophthalmus maximus* related to the specific time of the day. Data are mean concentrations in mmol / L ± SD, n=5

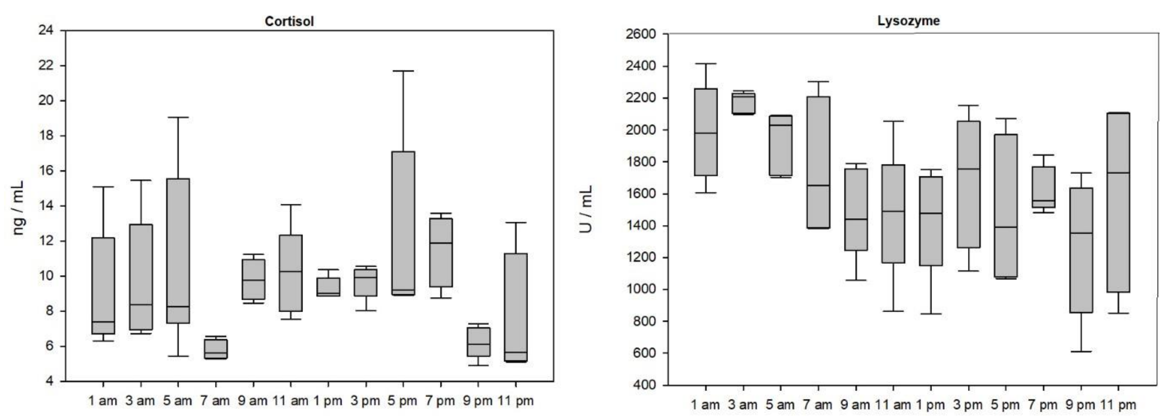

Figure 2 Metabolite levels in plasma of Scophthalmus maximus related to the specific time of the day. Data are mean concentrations in ng/ mL for cortisol and U/ mL for lysozymes ± SD, n=5

Metabolic profiling with NMR spectroscopy

46 metabolites could be identified in the ^{1}H-NMR spectra from blood plasma of turbot (see table 3 in the Annex). The number of metabolites did not changes between samples, but significant sampling-time dependent variations in metabolite concentrations could be observed. The One-Way ANOVA (p.value <0.05) and SAM (Delta = 0.3) results show significant differences for isoleucine, leucine, valine, phenylalanine, lysine, and lactate at 3 pm within a 24 hour period (Figure 3-8).

The main compounds found were osmolytes and amino acids. Organic osmolytes were taurine, betaine, methylamine, and trimethyl-N-oxide (TMAO). Free amino acids identified were alanine, asparagine, aspartate, glutamine, glutamate, glycine, homocysteine, isoleucine, L-arginine, leucine, lysine, methionine, ornithine, phenylalanine, tryptophan, and valine, as well as amino acid derivatives such as pyroglutamate, pipecolate, succinyl acetone, tyrosine, and uracil. Biotin, a vitamin, was found in very low concentrations, as was carnithine, which plays an important role in the synthesis of fatty acids and the metabolism of energy. Creatine, creatinine and creatine phosphate have also been identified, all of which are involved in the production of ATP. There were four compounds of the Krebs cycle identified in very low concentrations: Citrate, succinate, malate and fumarate. The Krebs cycle is the main source of energy for cells, and the compounds can be used as biomarkers for anaerobic metabolism. We also found glucose and pyruvate as indicators of glycolysis and energy storage. In addition, we identified one membrane-related intermediates, choline, and two sugars: maltose, sucrose, as well as the sugar alcohol mannitol. Metabolites involved in immune and stress responses found were histamine, lactate, O-acetylcholine, and serotonin. The singlet of acetate, involved in appetite in fish, was assigned despite an overlap with arginine and lysine.

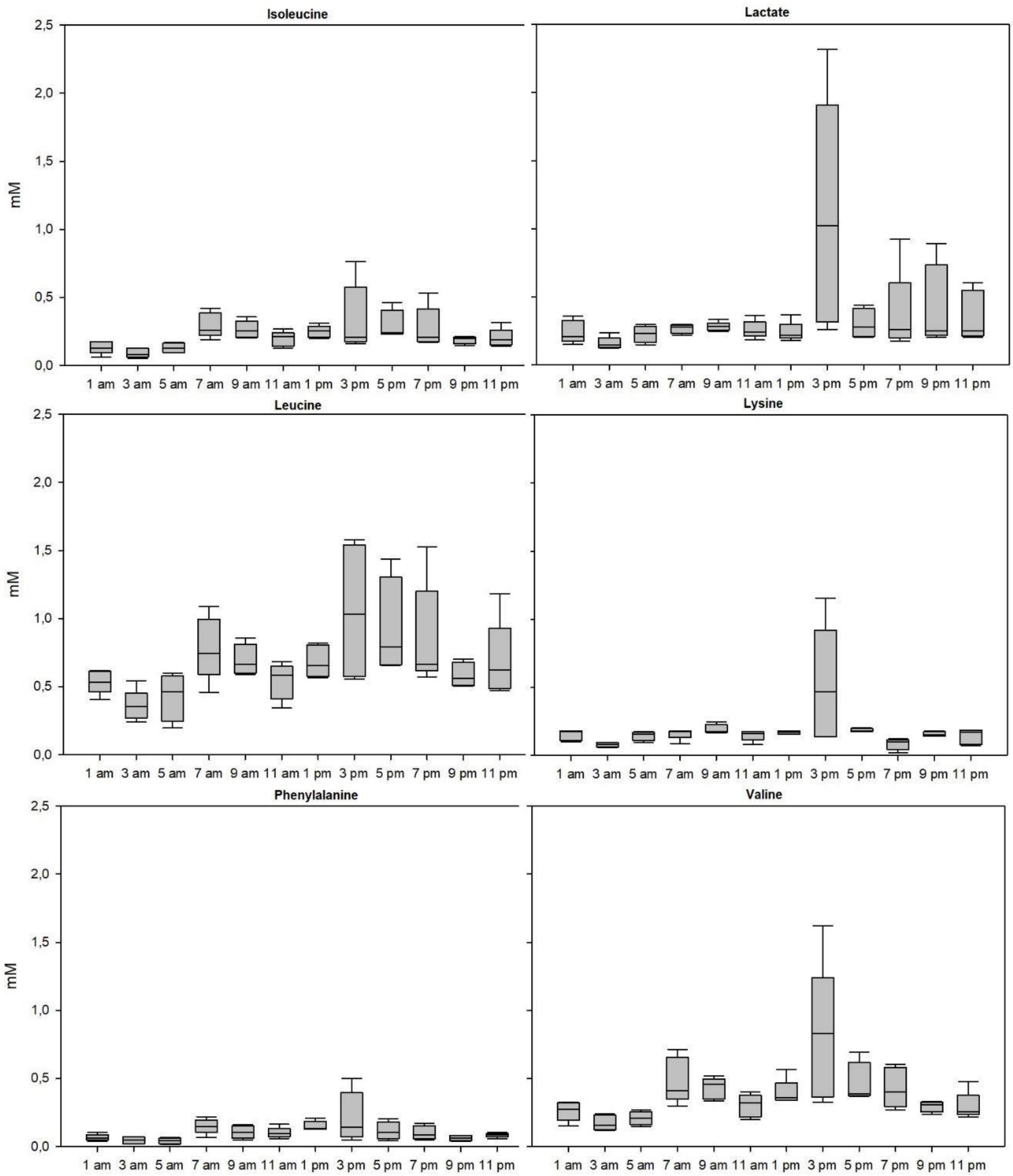

Figure 3 Metabolite levels in plasma of Scophthalmus maximus related to the specific time of the day. Data are mean concentrations in mM ± SD, n=5

Discussion

Metabolomics based on NMR spectroscopy in the study of body fluids such as plasma and serum blood represents an effective approach to elucidate the interactions between aquatic organisms and their environment (Samuelson et al., 2006), discover biomarker profiles (Macias et al., 2019), and gain deep insights into mechanisms associated with circadian rhythms in fish (Figueiredo et al. 2020; Lopes et al., 2022; Montero et al., 2019). Therefore, we applied untargeted metabolic profiling based on NMR spectroscopy to identify metabolic changes in turbot *Scophthalmus maximus* during a 24-hour period. To our knowledge, this is the first research looking at turbot in RAS to identify key metabolites over a 24-hour period. To better understand this complex issue, metabolites are divided into three categories: stress response, immune functions and amino acids.

Stress response

Cortisol, glucose, and lactate are well-known and well-established primary and secondary stress parameters, and the concentrations determined here are within the range of previously published work (Deborde et al., 2021; Foss et al., 2019; Hernández- Pérez et al., 2019 Imsland et al., 2008; López-Olmeda et al., 2009; Salamanca et al., 2020; van Ham et al., 2003). In the following, the individual parameters are discussed in more detail.

This study shows no significant differences in cortisol concentrations during the 24-h period examined, which is consistent with studies of gilthead sea bream (*Sparus aurata*), Senegalese sole (*Solea senegalensis*) (Costas et al., 2011) and trout (*Oncorhynchus mykiss*) (Hernández-Pérez et al., 2019; Montoya et al., 2010). However, somewhat lower levels were found at 7 am and 9 pm, shortly after lights were turned on and off, suggesting that overall stress levels appear to be lowest at these times. Other studies suggest no significant changes in cortisol levels over a 24-hour period, but a slight decrease shortly after lights are turned on and off, as in trout (Hernández-Pérez et al., 2019) or sea bream (López-Olmeda et al., 2009). As this is the first study to investigate cortisol levels in turbot over 24 hours, further studies are needed to confirm the results obtained here. Other studies have shown that cortisol levels follow a diurnal pattern and may indicate the time when fish are least stressed according to their rhythms (Figueiredo et al., 2020; Hernández-Pérez et al., 2019; Lopes et al., 2022; Montoya et al., 2010; Sánchez-Vázquez et al., 2019).

The glucose values in our study, both with test stripes and with NMR spectroscopy, show that turbot have a very stable baseline. This is not surprising, as other studies have indicated the same general baseline for glucose with similar values, as in trout, Senegalese sole or catfish (*Lophiosilurus alexandri*) (Costas et al., 2011; Figueiredo et al., 2020; Fortes-Silva et al., 2019; Hernández-Pérez et al., 2019; López-Olmeda et al., 2009). At 3 pm, a slight increase in glucose, maltose, and sucrose is observed in our results, which may indicate that fish have become accustomed to feeding at this time. Studies have shown that blood glucose levels of fish increase after their usual feeding time (Hernández-Pérez et al., 2019). It is interesting to note that we do not observe an increase at 9 am or 11 am, which is when the fish were normally fed for the first time of the day. This could possibly be due to the fact that all fish were starved for 24 hours before the start of the experiment, i.e., fish at 9 am were less starved than fish at 3 pm. Studies have shown that peak hormone and glucose activities are related to feeding cycles (Lopes et al., 2022). Thus, the 24-hour fasting period to which the animals were subjected in this study may have influenced the absence of significant rhythms for cortisol or blood glucose at 9 am. Thus, further experiments testing 24-hour fasting for all time points are needed to determine if this makes a difference.

Creatine is a naturally occurring non-protein compound whose primary metabolic role is to combine creatine with a phosphoryl group to generate phosphocreatine, which is used to regenerate adenosine triphosphate (ATP) (Borchel et al., 2014). This high intercorrelation can be well illustrated with our data, showing a direct dependence of creatine, creatinine and creatine phosphate. In addition, we detected a peak at 3 pm, as was also the case for the various sugars and some amino acids. Creatine contributes to the maintenance of energy supply and is therefore especially important in stressful situations and digestive phases (McFarlane et al., 2001). Studies have shown that creatine supplementation increases endurance in swimming tests in rainbow trout, but the effects of creatine supplementation are still underexplored for turbot (Borchel et al., 2014; McFarlane et al., 2001).

Overall, stress responses in fish are poorly understood and require further attention (Figueiredo et al., 2020). Nonetheless, identifying stress variations may be an interesting tool to minimize the effects of stressors in an aquaculture farm and thus improve fish welfare. Studies in gilthead sea bream and catfish have shown that feeding times influence the level of cortisol in relation to diurnal rhythms, further increasing the importance of underlying processes in identifying optimal species-specific feeding times (Fortes-Silva et al., 2019; Montoya et al., 2010).

Immunological response

Several studies suggest a strong link between the immune response and the circadian rhythm in fish, which is often influenced by light and feeding times (Costas et al., 2011; Montero et al., 2019; Schleiermann, 2013; Tort, 2011). However, in this study, no significant differences in the immunological parameters lysozyme, histamine, O- acetylcholine, or serotonin were detected within a 24-hour period in turbot. Lysozyme levels were within the range of other studies and were slightly higher between 1 am and 5 am, indicating the best immune functionality (Costas et al., 2011). This can be explained by the fact that the turbot were not stressed by light, feeding or handling at these times and were in a resting state.

In addition to assessing stress, lactate also serves as an important carbon source for various bacteria (Jääskeläinen et al., 2019). Consequently, high lactate levels in fish could increase bacterial infestation, leading to an increased risk of disease. A suggestion for future aquaculture operations could be to stop feeding at high lactate levels in turbot to prevent the growth of harmful bacteria. In the lactate test strips, a significantly higher difference in lactate levels was observed in turbot between 3 am and 9 am. However, it is questionable how accurate these values are. The concentrations are often below the threshold of the strips and the values could not be confirmed with NMR spectroscopy. Because glucose was detected in both the strips and NMR, we could interpret that the lactate levels were too low for determination with the strips and therefore could not be detected. The trend in NMR data of lactate cannot be reproduced with the lactate strips and must therefore be interpreted with caution. Further studies need to reproduce this trend, in which we found elevated lactate levels in the test strips between 3 am and 9 am, but elevated levels in NMR spectroscopy at 3 pm. Identifying the lowest immunological barriers is important to optimize feeding, transport, and handling in RAS and reduce the risk of disease outbreaks in aquaculture operations.

Amino acids

Amino acids (AA) are in strong correlation with stress and immune parameters and can help fish to better cope with stressful situations (Costas et al., 2011). All measured AA were within the range of fish and the most important ones will be analysed in more detail (Costas et al., 2011).

Branched-chain amino acids (BCAAs) are essential for regulating protein synthesis in skeletal muscle and are critical to fish physiology (Ahmad et al., 2021). BCAAs include isoleucine, valine, and leucine. All three BCAAs share the same intestinal transport and catabolic enzyme system, resulting in a high correlation. Both deficiency and excess of leucine and isoleucine in the diet can lead to reduced growth in many fish species and is therefore an important factor to consider when looking at circadian rhythms (Ahmad et al., 2021; Ahmed and Khan, 2006; Deng et al., 2014; Yamamoto et al., 2004). Optimization of BCAAs in fish diets is therefore critical for maximizing fish growth and health, and further studies are needed to investigate the BCAA requirements of turbot. In this study, a significant difference was found in BCAA concentrations, which first increase during the day (from 7 am to 7 pm, when it is light), then decrease again and shows the highest level at 3 pm. These results are supported by other studies who showed that light significantly affects the BCAA concentrations (Wu et al. (2021). Implementations for the increased concentrations at 3 pm could be to feed the animals during this period to optimize growth and digestion.

Besides the three BCAAs and lactate, were also phylalanine and lysine significantly increased at 3 pm. Phenylalanine is a glycogenic essential amino acid (EAA) and is responsible for many metabolic functions such as feed conversion, antioxidant capacity, or protein storage capacity (Xiao et al., 2020). Most aquaculture feeds include alternative protein sources to fishmeal and fishoil. Often are plant derived proteins introduced into feed formulations but do not meet the essential amino acid requirements for fish (Petereit et al., 2022; Xiao et al., 2020). A recent study by Salamanca et al. (2020) provided information that adding tyrosine, the precursor of phenylalanine, and phenylalanine to the diet leads to less stressed fish under typical stress conditions in an aquaculture facility. Our data shows peaks of both metabolites at 3 pm, which further studies need to confirm. Using this information and comparing it to the natural occurrence of phenylalanine in animals may improve fish welfare as well as feed utilization under standard aquaculture practices without adding expensive additives to the feed (Xiao et al., 2020.

Lysine is an another EAA and is usually the limiting factor in plant-based fish feed ingredients (Ebeneezar et al., 2019). Deficiency of lysine in the diet impairs the immunity of animals and increases their susceptibility to infectious diseases (Liao et al., 2015). In our study, as with many other essential AAs, a peak was detected at 3 pm, suggesting that this is a good time for feeding or handling. The natural occurrence of essential amino acids at 3 pm could promote feed conversion and thus fish growth and profitability. Stress conditions overall favour the mobilization of AA towards the fish brain and supplementations of AA could be used as energy substrates to better cope with stress (Costas et al., 2011; Salamanca et al., 2020). This study provides peak metabolic values for all investigated AA, which could be interpreted as the best time for handling and transportation.

Conclusion

Changing circadian rhythm in aquaculture farms is a growing concern that impacts health, food safety, and farm profitability. In our comprehensive study, we are creating and analysing the first dataset of its kind to better understand the circadian rhythm of turbot. The data collected can help optimize rearing protocols and improve the welfare of turbot on fish farms.

This study showed some differences and trends in the circadian rhythm in the metabolites of turbot kept in RAS, analysed with NMR spectroscopy. As a result, 46 metabolites were identified, eight of which appeared to shift throughout the day. We noted an increase of metabolites around 3 pm, including several sugars, like glucose and sucrose as well as multiple amino acids like phenylalanine, leucine, isoleucine, valine and lysine. All are known as energy suppliers and using times of high metabolic rates could help decrease the affection of stressful situations in fish. We saw furthermore no significant changes in the immune response over the day, neither in lysozymes nor cortisol. If future studies confirm our trend at high energy supplying metabolites at 3 pm, like glucose, and several amino acids, farm management strategies could be optimized to decrease stress in fish and therefore optimize growth and health without further expenses. This would not only have a positive impact on farm productivity but also lead to significant improvements in economic and animal welfare terms.

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Annex

Table 2: Plasma metabolites n = 5 given as mean values with standard deviation. Grey highlighted numbers represent the timepoint with the highest concentration for that metabolite

Time / Metabolite (mM)	1:00 AM	3:00 AM	5:00 AM	7:00 AM	9:00 AM	11:00 AM	1:00 PM	3:00 PM	5:00 PM	7:00 PM	9:00 PM	11:00 PM
Glucose (mmol/L)	3.00	3.02	2.62	2.86	2.64	2.58	3.30	3.08	2.58	3.10	2.62	2.63
± SD	0.77	0.61	0.50	0.98	0.50	0.46	0.51	0.81	0.56	0.61	0.57	0.46
Lactate (mmol/L)	bT	1.56	1.50	1.00	bT	bT	bT	bT	bT	0.90	bT	bT
± SD		0.12	0.20	0.00						0.00		
Lysozyme (U/mL)	1983.09	2174.05	1926.73	1748.52	1384.39	1477.27	1438.55	1677.73	1498.27	1625.36	1267.27	1606.08
± SD	271.46	59.63	174.34	386.78	259.88	378.09	316.82	373.86	407.74	128.75	388.96	526.94
Cortisol (ng/mL)	9.23	9.83	10.50	5.66	9.86	10.17	9.24	9.63	12.48	11.54	6.14	7.74
± SD	3.45	4.00	5.11	1.09	2.20	3.53	0.97	1.41	5.41	2.15	1.25	3.68

Legend: bT -> below Threshold

Table 3 Plasma Metabolites (n=5) in mM given as mean values ± SD at the different timepoints. Grey highlighted numbers represent the timepoint with the highest concentration for that metabolite

Time / Metabolite (mM)	1:00 AM	3:00 AM	5:00 AM	7:00 AM	9:00 AM	11:00 AM	1:00 PM	3:00 PM	5:00 PM	7:00 PM	9:00 PM	11:00 PM
Acetate	**0.408**	**0.293**	**0.387**	**0.438**	**0.459**	**0.405**	**0.400**	**1.102**	**0.616**	**0.491**	**0.469**	**0.443**
± SD	0.055	0.043	0.015	0.056	0.073	0.088	0.046	0.744	0.273	0.370	0.091	0.091
Alanine	**0.260**	**0.154**	**0.229**	**0.247**	**0.261**	**0.271**	**0.253**	**0.479**	**0.369**	**0.295**	**0.473**	**0.299**
± SD	0.091	0.032	0.065	0.056	0.039	0.059	0.047	0.284	0.202	0.188	0.278	0.089
Asparagine	**0.198**	**0.115**	**0.103**	**0.071**	**0.194**	**0.080**	**0.066**	**0.130**	**0.171**	**0.054**	**0.060**	**0.154**
± SD	0.184	0.149	0.115	0.033	0.231	0.032	0.046	0.113	0.121	0.031	0.048	0.193
Aspartate	**0.189**	**0.038**	**0.190**	**0.104**	**0.114**	**0.065**	**0.070**	**0.526**	**0.207**	**0.234**	**0.098**	**0.148**
± SD	0.165	0.034	0.141	0.108	0.085	0.055	0.064	0.706	0.117	0.403	0.074	0.200
Betaine	**0.040**	**0.015**	**0.022**	**0.034**	**0.023**	**0.027**	**0.047**	**0.064**	**0.036**	**0.048**	**0.030**	**0.044**
± SD	0.024	0.009	0.013	0.014	0.007	0.015	0.014	0.035	0.021	0.039	0.008	0.026
Biotin	**0.050**	**0.037**	**0.061**	**0.072**	**0.089**	**0.060**	**0.083**	**0.104**	**0.092**	**0.074**	**0.058**	**0.060**
± SD	0.001	0.020	0.029	0.002	0.029	0.021	0.050	0.080	0.068	0.006	0.013	0.022
Carnitine	**0.002**	**0.026**	**0.017**	**0.004**	**0.030**	**0.029**	**0.026**	**0.089**	**0.035**	**0.004**	**0.023**	**0.026**
± SD	0.013	0.013	0.010	0.004	0.006	0.012	0.006	0.070	0.017	0.030	0.016	0.012
Choline	**0.031**	**0.023**	**0.024**	**0.030**	**0.004**	**0.026**	**0.022**	**0.034**	**0.003**	**0.029**	**0.020**	**0.035**
± SD	0.011	0.005	0.009	0.011	0.035	0.013	0.008	0.021	0.011	0.013	0.006	0.027
Citrate	**0.059**	**0.049**	**0.041**	**0.066**	**0.068**	**0.043**	**0.043**	**0.070**	**0.058**	**0.044**	**0.044**	**0.056**
± SD	0.035	0.044	0.008	0.038	0.040	0.020	0.009	0.047	0.026	0.035	0.024	0.026
Creatine	**0.076**	**0.055**	**0.070**	**0.080**	**0.086**	**0.062**	**0.057**	**0.281**	**0.145**	**0.069**	**0.069**	**0.089**
± SD	0.018	0.034	72.100	0.028	0.029	0.026	0.013	0.282	0.071	0.020	0.025	0.041

Creatine phosphate	0.141	0.084	0.114	0.136	0.147	0.102	0.121	0.461	0.236	0.125	0.105	0.169
± SD	0.021	0.050	0.030	0.048	0.039	0.026	0.060	0.522	0.139	0.048	0.036	0.125
Creatinine	0.069	0.052	0.052	0.098	0.082	0.054	0.068	0.130	0.115	0.046	0.051	0.077
± SD	0.010	0.027	0.011	0.030	0.017	0.026	0.034	0.136	0.075	0.024	0.017	0.051
Fumarate	0.002	0.001	0.003	0.002	0.002	0.002	0.003	0.004	0.002	0.003	0.002	0.002
± SD	0.001	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.001	0.001	0.001	0.001
Glucose	1.978	1.930	1.599	1.965	1.994	1.951	1.968	3.641	2.920	2.038	1.872	2.050
± SD	0.534	0.572	0.339	0.501	0.355	0.701	0.940	2.472	1.220	0.877	0.271	0.315
Glutamate	0.246	0.166	0.222	0.254	0.272	0.234	0.247	0.608	0.310	0.361	0.240	0.247
± SD	0.032	0.092	0.058	0.061	0.074	0.051	0.078	0.524	0.107	0.244	0.059	0.127
Glutamine	0.120	0.083	0.133	0.136	0.151	0.087	0.122	0.209	0.145	0.124	0.152	0.111
± SD	0.037	0.042	0.024	0.019	0.031	0.015	0.050	0.164	0.052	0.102	0.037	0.070
Glycine	0.223	0.204	0.192	0.249	0.273	0.223	0.216	0.595	0.280	0.264	0.255	0.303
± SD	0.064	0.051	0.058	0.044	0.122	0.077	0.052	0.466	0.080	0.200	0.120	0.158
Histamine	0.010	0.008	0.013	0.015	0.012	0.011	0.012	0.018	0.009	0.008	0.007	0.008
± SD	0.004	0.001	0.007	0.014	0.004	0.003	0.005	0.006	0.001	0.003	0.001	0.001
Homocysteine	0.084	0.031	0.039	0.086	0.060	0.034	0.046	0.096	0.050	0.058	0.041	0.071
± SD	0.095	0.019	0.014	0.115	0.069	0.026	0.018	0.117	0.033	0.062	0.021	0.040
Isoleucine	0.134	0.092	0.129	0.294	0.263	0.196	0.249	0.343	0.292	0.278	0.188	0.204
± SD	0.048	0.033	0.037	0.090	0.064	0.054	0.044	0.251	0.114	0.152	0.028	0.067
Lactate	0.241	0.159	0.225	0.265	0.281	0.258	0.241	1.093	0.300	0.372	0.400	0.355
± SD	0.082	0.045	0.061	0.032	0.034	0.067	0.073	0.849	0.108	0.310	0.329	0.181
L-Arginine	0.482	0.377	0.451	0.527	0.623	0.491	0.524	0.706	0.595	0.455	0.310	0.521
± SD	0.131	0.153	0.196	0.118	0.128	0.141	0.103	0.370	0.122	0.098	0.130	0.184
Leucine	0.537	0.359	0.430	0.783	0.698	0.542	0.685	1.050	0.918	0.860	0.583	0.691
± SD	0.085	0.114	0.176	0.233	0.112	0.133	0.117	0.532	0.364	0.389	0.090	0.288
Lysine	0.149	0.078	0.145	0.158	0.192	0.147	0.168	0.516	0.183	0.083	0.155	0.139
± SD	0.039	0.017	0.034	0.040	0.033	0.039	0.012	0.426	0.013	0.041	0.017	0.056
Malate	0.111	0.087	0.098	0.108	0.142	0.098	0.103	0.166	0.149	0.094	0.083	0.110
± SD	0.032	0.050	0.025	0.010	0.044	0.038	0.015	0.091	0.064	0.055	0.031	0.028
Maltose	0.107	0.120	0.064	0.087	0.163	0.042	0.070	0.227	0.069	0.056	0.067	0.171
± SD	0.080	0.106	0.020	0.077	0.216	0.013	0.048	0.183	0.019	0.022	0.025	0.236
Mannitol	0.579	0.442	0.553	0.627	0.667	0.657	0.786	1.830	0.946	0.700	0.841	0.794
± SD	0.256	0.144	0.169	0.284	0.120	0.144	0.051	1.230	0.521	0.463	0.241	0.185
Methionine	0.061	0.043	0.082	0.092	0.106	0.076	0.109	0.218	0.135	0.097	0.085	0.114
± SD	0.024	0.029	0.027	0.033	0.040	0.032	0.037	0.177	0.094	0.092	0.021	0.027
Methylamine	0.052	0.034	0.049	0.046	0.055	0.032	0.035	0.094	0.058	0.045	0.037	0.051
± SD	0.029	0.029	0.031	0.021	0.033	0.007	0.012	0.092	0.023	0.055	0.011	0.033
O-Acetyl choline	0.038	0.032	0.027	0.042	0.031	0.029	0.037	0.098	0.039	0.045	0.025	0.036
± SD	0.009	0.011	0.006	0.008	0.003	0.005	0.027	0.077	0.009	0.024	0.009	0.012
Ornithine	0.074	0.070	0.069	0.074	0.070	0.060	0.060	0.061	0.069	0.063	0.063	0.068
± SD	0.005	0.025	0.010	0.008	0.009	0.021	0.026	0.020	0.009	0.016	0.013	0.012
Phenylalanine	0.065	0.045	0.041	0.148	0.106	0.100	0.151	0.214	0.114	0.101	0.062	0.084
± SD	0.024	0.025	0.023	0.055	0.048	0.040	0.035	0.183	0.067	0.051	0.020	0.016
Pipecolate	0.070	0.053	0.103	0.090	0.113	0.142	0.067	0.354	0.125	0.137	0.147	0.078

± SD	0.011	0.058	0.023	0.023	0.052	0.123	0.023	0.491	0.052	0.159	0.074	0.027
Pyro glutamate	0.086	0.084	0.102	0.142	0.130	0.100	0.145	0.112	0.144	0.142	0.076	0.100
± SD	0.042	0.077	0.052	0.017	0.016	0.023	0.093	0.036	0.024	0.111	0.031	0.040
Pyruvate	0.029	0.022	0.028	0.044	0.036	0.035	0.028	0.140	0.043	0.039	0.034	0.039
± SD	0.009	0.011	0.014	0.013	0.011	0.016	0.009	0.163	0.026	0.018	0.013	0.032
Serotonin	0.037	0.031	0.037	0.051	0.070	0.049	0.044	0.085	0.046	0.050	0.043	0.034
± SD	0.012	0.009	0.010	0.010	0.030	0.011	0.006	0.076	0.014	0.012	0.008	0.013
Succinate	0.017	0.013	0.015	0.028	0.020	0.013	0.016	0.070	0.020	0.016	0.018	0.018
± SD	0.005	0.007	0.007	0.008	0.007	0.005	0.006	0.073	0.015	0.007	0.008	0.012
Succinyl acetone	0.043	0.024	0.044	0.056	0.048	0.034	0.045	0.037	0.046	0.031	0.044	0.040
± SD	0.003	0.010	0.009	0.022	0.016	0.015	0.010	0.019	0.005	0.010	0.014	0.008
Sucrose	0.035	0.016	0.011	0.009	0.069	0.010	0.012	0.060	0.018	0.019	0.012	0.032
± SD	0.031	0.010	0.003	0.004	0.134	0.005	0.006	0.052	0.009	0.016	0.005	0.037
Taurine	0.083	0.129	0.091	0.091	0.072	0.077	0.104	0.079	0.060	0.126	0.073	0.101
± SD	0.020	0.049	0.013	0.046	0.028	0.032	0.052	0.029	0.038	0.070	0.016	0.034
Trimethyl amine N-oxide	0.025	0.012	0.017	0.025	0.025	0.018	0.032	0.037	0.021	0.042	0.017	0.016
± SD	0.014	0.006	0.006	0.006	0.010	0.012	0.017	0.030	0.008	0.056	0.005	0.009
Tryptophan	0.015	0.014	0.023	0.034	0.020	0.017	0.027	0.022	0.015	0.023	0.013	0.018
± SD	0.004	0.004	0.013	0.027	0.005	0.006	0.016	0.014	0.006	0.010	0.003	0.003
Tyramine	0.029	0.027	0.035	0.059	0.058	0.026	0.042	0.081	0.045	0.039	0.034	0.044
± SD	0.012	0.013	0.009	0.020	0.026	0.014	0.022	0.090	0.041	0.020	0.026	0.015
Tyrosine	0.037	0.045	0.048	0.048	0.045	0.070	0.073	0.088	0.066	0.059	0.036	0.057
± SD	0.021	0.024	0.037	0.010	0.016	0.025	0.036	0.067	0.029	0.037	0.009	0.007
Uracil	0.008	0.006	0.008	0.008	0.008	0.010	0.009	0.017	0.009	0.007	0.008	0.007
± SD	0.002	0.001	0.003	0.004	0.001	0.001	0.001	0.013	0.002	0.000	0.001	0.001
Valine	0.259	0.171	0.207	0.483	0.428	0.301	0.394	0.808	0.459	0.430	0.295	0.294
± SD	0.072	0.055	0.050	0.167	0.078	0.084	0.096	0.515	0.157	0.147	0.040	0.103

Chapter VI

Synoptic discussion

Synoptic discussion and future recommendations

The dissertation offers recommendations to improve sustainable aquaculture consumption and production in Europe. The focus of my thesis is on analysing general consumer perceptions (**Chapter III**), identifying novel and more sustainable diets for European seabass (*Dicentrarchus labrax)* while ensuring profitability and fish welfare (**Chapter IV**), and identifying a general stress physiological approach aimed at determining differences in a 24-hour period for turbot (*Scophthalmus maximus*) to optimise aquaculture practices (**Chapter V**).

In order to answer the research questions formulated in 1.6. (**Chapter I**), the above mentioned chapters will be addressed in the following sections: 6.1, (**Chapter III**); 6.2, (**Chapter IV**); and 6.3, (**Chapter V**). Section 6.4 elaborates the practical applicability of the data and provides implications to the aquaculture sector. Likewise, section 6.5 addresses the limitations of these studies and provides future recommendations and 6.6 gives a comprehensive concusion of my work.

Consumer perception of seafood products in Europe

Sustainable food in general has gained significant importance in recent decades and has increasingly appeared on supermarket shelves (Banovic et al., 2019; Risius et al., 2019). However, the share of sustainable seafood products, especially fish from aquaculture, remains very low due to high production costs and poor consumer perception (Gambelli et al., 2019; Maesano et al., 2020). The first objective in **Chapter III** aims to understand the impact of increased scientific knowledge and country-specific differences on purchasing behaviour. The hypothesis stated that increased scientific knowledge about aquaculture led to increased purchases of sustainable seafood. To this end, the scientific expertise of participants from eight international knowledge transfer events (KTEs) was analysed using an interactive poster approach. A KTE comprised either a conference, a seminar, or a meeting attended by scientists, stakeholders, and aquaculture farmers.

The interactive poster approach is a new methodology not widely used in social sciences. However, this method seems to have great potential for the future since it can easily be combined with semi-structured interviews. Under this approach, participants self-reported on an A1 poster their preferences regarding seafood purchase criteria and preferred fish species (see Annex 1 and 2).

The fish species displayed comprised Atlantic salmon (*Salmo salar*) and gilthead sea bream (*Sparus aurata*) to freshwater rainbow trout (*Oncorhynchus mykiss*). These three species represent the most commonly cultivated fish species in Europe in terms of value and production volume (de la Casa-Resino et al., 2021; EUMOFA, 2019; EUMOFA, 2021; FAO, 2018; FEAP, 2020). Moreover, these species were the central candidates for circular economy approaches in the GAIN project (Hoerterer et al., 2022b; Petereit et al., 2022b). For the poster survey in Spain, however, trout was exchanged for European seabass (*Dicentrarchus labrax*), as this species is more common in southern Europe and also served as a central species for the feed trials in the GAIN project (Llorente et al., 2020; Muñoz-Lechuga et al., 2018; Petereit et al., 2022b; Rigos et al., 2020).

Based on an extensive literature review, I identified several criteria with the greatest influence in individual purchasing decisions: price, health benefits, certification importance, animal welfare, and product processing (Cantillo et al., 2021; Kreˇsi'c et al., 2020; Menozzi et al., 2020; Pulcini et al., 2020). The posters concentrated on these five criteria in order to make the survey as simple and accessible as possible. Vis a' vis to gain initial insight into the differences in terms of the level of scientific knowledge available and their respective influence on individual decision making.

The posters were presented at the various knowledge transfer events in a frequently accessed area. Semi-structured interviews were randomly conducted alongside to elicit their choices. In total, 383 participants indicated their preferences anonymously on the posters. Furthermore, QR codes were offered on the posters, as well as on additional flyers, which allowed participants to respond online to the surveys. Online questions were identical to those on the posters.

My work demonstrates that KTE participants appear to prefer poster over online surveys, indicating that a direct approach tends to be more successful than passive surveys. Moreover, the semi-structured interviews performed in parallel with the posters revealed complementary information that could not be captured by online surveys. Both of these aspects highlight that interactive posters hold great potential for the future, particularly for similar KTEs.

The KTEs took place in different countries, with three countries being identified individually: Spain, Poland, and Germany. The participants in these three KTE events were exclusively from the respective countries. The other five KTEs had international participants, and no country could be clearly identified. Semi-structured interviews helped to identify individuals at the international conferences and to gain further insight into country-specific purchasing behaviour. Semi-structured interviews are a commonly used method to obtain a deeper understanding about decision criteria (Bearman, 2019; Dale & Kline, 2017). These semi-structured interviews clarified the findings, took place in an informal tone, and served to gain more insight into the participants' decision-making (Bearman, 2019; Longhurst, 2004).

My surveys revealed country-specific differences regarding seafood consumption and purchasing criteria. From my results, I found that southern European countries, for example, Spain and Portugal, showed more interest in freshly caught local products rather than the fish species themselves. In southern European countries, traditional fishing practices and personal background may explain this purchasing behaviour (Jacobs et al., 2015; Menozzi et al., 2020). People may have been raised buying foods at local markets and accustomed to fresh fish from an early age. On the other hand, both German and Norwegian consumers favoured salmon and rarely reported buying any other type of fish. In fact, my results showed that only 8% of German participants reported eating fish species other than salmon, trout, or sea bream. This is confirmed by other studies, which have shown that German consumers prefer those species to which they are accustomed and that processed foods are more likely to be purchased than fresh, whole fish (Koch et al., 2019; Menozzi et al., 2020). Other purchase criteria such as price, animal welfare, health characteristics, and certification seemed to be equally important for all countries, and no differences were found in my surveys. Altogether, my findings revealed strong differences between northern and southern European countries regarding the species and type of processing, highlighting the need to adapt marketing campaigns at the national level.

The posters also revealed that people disassociated with the aquaculture sector had only limited knowledge in this field. My work focused exclusively on scientific knowledge; therefore, only scientific events were surveyed. My motivation was to specifically examine scientific knowledge of aquaculture practices to determine if a lack of education leads to a more negative attitude towards seafood. Based upon the results, my work show that the general perception of aquaculture increased with increased scientific knowledge. These findings corroborate previous studies and indicate that raising consumer awareness and education is key to shifting perceptions and, consequently, purchasing behaviours. (Almeida et al., 2015; Funk et al., 2021; Lawley et al., 2019; Maesano et al., 2020; Pulcini et al., 2020).

The semi-structured interviews also revealed that the concept of circular economy and RAS, as a rearing system, are largely unknown among less well-informed consumer groups. However, these management strategies can help promote sustainable production and improve consumer perceptions of aquacultured fish (Aubin et al., 2017; Hackenesch et al., 2016; Kirchherr et al., 2017). In addition, participants with higher levels of scientific aquaculture-specific knowledge were more aware of the positive attributes of aquaculture and how these attributes can help promote sustainable consumption. These findings confirm the importance of knowledge about purchasing behaviour and reasons why this area needs to be addressed to shift consumer perceptions (Gambelli et al., 2019; Jonell et al., 2016; Szendrő et al., 2020).

My work has also demonstrated that the younger generation in particular tends to adopt a vegan and vegetarian lifestyle, consistent with recent literature (Saari et al., 2021). Besides personal preferences and negative prejudices towards seafood, animal welfare, health, and sustainability play a crucial role in the decision for a vegan or vegetarian lifestyle (Govaerts, 2021; Menozzi et al., 2020). The misconception of aquaculture products may be associated with the reinforcement of a biased media image (Wongprawmas et al., 2022). Media in most cases prevail with negative portrayal of harmful aquaculture systems, thus ignoring current efforts and achievements towards sustainable aquaculture production systems (Zajicek et al., 2021). This was substantiated by my semi-structured interviews, where many of the younger participants (aged around 20–30 years) stated that they had watched TV shows, such as the Netflix documentary “Seaspiracy”, which conveyed a negative image of present-day aquaculture and fisheries (Pauly, 2021; Zajicek et al., 2021).

Product labelling might be a good way to increase consumer awareness of aquaculture products and counteract the negative reporting (Richter & Klöckner, 2017; Maesano et al., 2020). However, the semi-structured interviews revealed that many consumers are overwhelmed by the sheer number of market labels. The finding is consistent with other studies that point to both a lack of label transparency and emerging consumer confusion, which reduces trust in labelling and thus weakens its effectiveness in promoting more sustainable seafood purchasing patterns (Cantillo et al., 2021; Donlan & Luque, 2019; Richter & Klöckner, 2017; Risius et al., 2017).

In the semi-structured interviews, consumers with a higher level of scientific knowledge indicated a preference for seafood that comes from a close environment and for which the rearing conditions are stated on the product. Labelling systems that indicate both the production system (e.g. RAS) and the food origin might be a viable alternative to ambiguous labels. A clear indication of this on the packaging could steer consumers’ purchasing preferences more towards regionality (Altintzoglou et al., 2011; Risius et al., 2019).

The choice towards aquatic farmed animals does not only affect the environment (depending on the rearing system) but also the health attributes for consumers. Focusing more on explaining why fish from aquaculture are often safer than fishery products could help to shift the consumer attitude (Cantillo et al., 2021; Jacobs et al., 2018). Marine wild-caught fish accumulate biohazards from the sea in the filet and hence are often more toxic than species raised in RAS (Banovic et al., 2019; Schlag & Ystgaard, 2013; Thong & Solgaard, 2017). Consumers need to be educated about the environmental sustainability of their product (e.g. with a life cycle assessment), as well as its nutritional benefits, to gain a better understanding and to allow them to make purchase decisions on their own (Boyd et al., 2022; Legeza et al., 2019; Menozzi et al., 2020). Today’s society has evolved into a multi-layered and diverse entity with different interests, views, knowledge structures, perspectives, norms and values (Jacobs et al., 2015). These are also reflected in the decision-making when purchasing seafood. Preferences are highly dependent on a range of individual, cultural, and contextual factors, which contradict the positive role of scientific knowledge in a healthy information society.

My findings suggest that an information-based strategy focusing on both the product's origin and its sustainability could be an effective tool to shift consumer awareness and associated perceptions. An important note for existing campaigns is to tailor their products to the target country. This work and other studies have shown that perceptions and preferences are highly country-specific (Menozzi et al., 2020; Pieniak et al., 2008; Risius et al., 2019).

Sustainable fish feed concepts

Intensive aquaculture offers a promising solution to meet global protein demand, achieve the SDGs, and address malnutrition and hunger (Balami et al., 2019; Boyd et al., 2022; FAO, 2020; Fonseca et al., 2020; Henchion et al., 2017; Komarnytsky et al., 2022). However, increasing fish production in aquaculture is associated with high protein content in fish feed, usually from unsustainable sources (Gerten et al., 2020; Luthada- Raswiswi et al., 2021; Owsianiak et al., 2022; Willer et al., 2022).

Objective 2, discussed in **Chapter IV**, focusses on sustainable feed concepts that could replace commercial feed for adult European seabass (*Dicentrarchus labrax*) in RAS. European seabass is a carnivorous species mainly reared in intensive aquaculture, making this species well-researched and highly suitable for identifying new feed concepts (FAO, 2022; FEAP, 2017; Llorente et al., 2020; Rigos et al., 2020; Vandeputte et al., 2019). My work investigates whether alternative ingredients, such as hydrolysates from the circular economy, plant and animal derived proteins, as well as insects, can replace fishmeal and fishoil without compromising the health and growth of individual fish. European manufactured and circular-economy-derived products have been used to promote blue growth and reduce dependency on other countries and unsustainable resources.

Based on the formulation of commercial seabass diets, four alternative iso-nitrogenous feed concepts were designed and tested in a long-term feeding trial. The alternative diets replaced commercial fishmeal and fishoil with fish hydrolysates from aquaculture, insectmeal, and animal- and plant-derived proteins. The percentage of fishmeal and fishoil could be reduced from 23.5% to 5.7%; the complete list of ingredients can be found in Annex 3. The main performance parameters included fish health, growth, sensory attributes, and animal welfare characteristics.

Neither growth, health, nor sensory properties, including taste and odour, altered significantly. Furthermore, chemical composition and nutrient analysis revealed no significant changes between the alternative diets and the fish-fed the control diet. Blood parameters, which are intended to indicate animal welfare, were inconspicuous as well. Lysozyme levels as well as glucose and lactate dehydrogenase were within the normal range reported for this species and showed no differences, indicating good welfare.

Besides welfare, the growth parameter is the most important factor for the farmer, especially for determining the feed's efficiency and profitability. The most promising variables used for this purpose are the feed conversion ratio (FCR) and protein efficiency ratio (PER) (Alam et al., 2009; Besson et al., 2020; Siddik et al., 2021; Omasaki et al., 2017). Neither PER nor FCR revealed any adverse effects for fish-fed the commercial diet compared to fish-fed the diet containing more plant-derived proteins.

Due to the described factors, finding sustainable protein sources for aquaculture feed is critical for shifting and reducing resource pressure. The use of aquaculture by-products has many advantages compared to commercial FM. They involve much shorter transport distances (as they are usually reused on site) and recycle existing resources, which makes production more environmental friendly, overall. These results suggest that plant-based ingredients and aquaculture by-products could contribute to more sustainable feed design and promote ecological intensification. Accordingly, the best solution for future seabass diets would be to replace FM with hydrolysates and SBM, with more sustainable crops, such as wheat and enriched algae, as well as adding agricultural by- products, such as feather meal for better mineral composition and digestibility. This transfer could positively influence consumer attitudes towards more farmed fish, provide greater incentive for farmers to adopt more environmental friendly measures, and support industrial growth with a focus on circular economy.

The circadian rhythm of turbot in RAS

Increasing public awareness and the impact of farming practices has led to intensified research in fish welfare. Moreover, consumers often state that animal welfare, along with price and sustainability, influences their purchasing behaviour the most (Ankamah-Yeboah et al., 2019; Funk et al., 2021, Maesano et al., 2020). This hypothesis could be further validated by the findings from **Chapter III**. Increasing fish welfare is hence essential to improve eco-intensification and make aquaculture products more appealing to consumers.

Towards the end of my dissertation, I studied the metabolic processes over 24 hours of turbot (*Scophthalmus maximus*) culture in RAS (**Chapter V**). This species is well-studied in terms of its growth parameters. However, information on its stress physiology remains scarce. To the best of my knowledge, this has been the first study that examined the CR of farmed turbot. Research objective 3 aimed to identify metabolites with NMR spectroscopy that changed over a 24-hour period. Such information could help optimise aquaculture practices and consequently increase productivity through better welfare.

A total of 46 metabolites in the plasma was detected and qualified by NMR spectroscopy. My results indicate that many of the metabolites peak at 3 p.m., which may be interpreted either as habituation to the usual feeding regime or natural metabolic peaks. The animals were habituated to feeding times at 9 a.m. and 3 p.m., six months prior to the experiment. However, the fact that there is only one peak at 3 p.m. and no evidence at 9 a.m. suggests a natural metabolic peak. Natural metabolic peaks are determined by the metabolism of the animal as well as through clock genes and are highly species-specific, as well as vary over a 24-hour period (Richards & Gumz, 2013; Cowan et al., 2017; Sánchez-Vázquez et al., 2019). My findings indicate higher metabolic levels of isoleucine, lactate, leucine, lysine, phenylalanine and valine at 3 p.m. in particular. A closer look at these metabolites reveals that isoleucine, leucine, and valine make up the group of branched-chain amino acids (BCAAs) and are essential for modulating protein synthesis and exhibiting light sensitivity (Ahmand et al., 2021; Wu et al., 2021). Proteins are the most important component of fish diets because they promote growth, health, energy, and well-being, making BCAAs special metabolites for intensive fish production (Andersen et al., 2016; Kawanago et al., 2015). Moreover, my results confirm the light sensitivity of BCAAs, as their content in turbot increases during the day and decreases with nightfall (Wu et al., 2021). Optimisation of feeding times to the metabolic needs of fish can stimulate feed intake and utilisation (Assan et al., 2021). Moreover, avoiding feed waste through proper feeding minimizes the loss of expensive feed and the accumulation of nitrogenous waste in the water, which could be toxic to the fish (Assan et al., 2021). As a result, this coud contribute to reducing the amount of protein required in the diets and support the profitability of the farm.

Amino acids (AA) are furthermore in strong correlation with stress and immune parameters and could help fish to better cope with stressful situations inside the farm (Costas et al., 2011). My findings revealed increased values in many diverse AA at 3 pm, which could be interpreted as the best time for handling and transportation. Previos studies concluded that stress conditions overall favour the mobilization of AA towards the fish brain and supplementations of AA could be used as energy substrates to better cope with stress (Costas et al., 2011; Salamanca et al., 2020).

Cortisol, glucose, and lactate are well-known and well-established primary and secondary stress parameters, and the concentrations determined here are within the range of previously published work (Deborde et al., 2021; Foss et al., 2019; Hernández- Pérez et al., 2019 Imsland et al., 2008; López-Olmeda et al., 2009; Salamanca et al., 2020; van Ham et al., 2003). Glucose and cortisol did not show significant differences during the 24-h period, which is consistent with recent literature, which showed a stable baseline for both metabolites in trout, catfish and senegalese sole (Costas et al., 2011; Figueiredo et al., 2020; Fortes-Silva et al., 2019; Hernández-Pérez et al., 2019; Montoya et al., 2010). Immune parameters like lysozyme, histamine, O-acetylcholine and serotonin showed a similar picture and did not vary significantly over a 24 h period in turbot.

If future studies confirm my findings of high metabolic rates in sugar and amino acids at 3 p.m., management strategies can be optimised and help improve economic and welfare conditions to increase ecological intensification of intensive fish farming. To maximise aquaculture productivity, abiotic factors such as light conditions and temperature are often manipulated in farms to increase yield (Ceinos et al., 2019; Sánchez-Vázquez et al., 2019; Santos et al., 2021). In particular, light stimuli disrupt circadian rhythms and can therefore interfere with the animals' physiological processes

(Choi et al., 2022; Lee et al., 2021; Richards & Gumz, 2013; Sánchez-Vázquez et al., 2019). This disruption can lead to an increase in stress and disease, as well as a decline in welfare, which is important for intensive fish farming (Brochu et al., 2021; Saiz et al., 2021). Studies have also shown that feeding at random or non-optimal times can lead to lower growth and welfare of animals (Boujard & Leatherland, 1992; Gilannejad et al., 2019; Lopes et al., 2022; Saiz et al., 2021). Consequently, quantifying the data and identifying times of day when stress levels are high will aid in suggesting feeding regimes to support the natural metabolism of fish. The here found peaks at 3 p.m. in sugars and amino acids for instance, could be used as energy stores and help fish to better cope with stressful farm activities. By adjusting handling and feeding regimes to species- specific circadian rhythms, stress can be reduced, and the farmer could increase productivity without additional costs.

Implications for aquaculture practices

My dissertation was about finding and developing ways to improve the sustainability of intensive aquaculture farms by increasing positive consumer perceptions and fish welfare, as well as through the application of more sustainable feeds. I found that the first step in promoting the consumption of aquaculture fish should be to shift consumer attitudes. Consumers are the most critical factor that limit sustainable farmed fish products in the marketplace, as supply and demand determine its presence on the shelf (Bronnmann & Asche, 2017; Culliford & Bradbury, 2020; Koch et al., 2019; Menozzi et al., 2020). Hence, the demand for aquaculture fish has to increase in order to lead the way to more sustainable production.

Chapter III has demonstrated that both consumer awareness and perception are linked to their knowledge of the fish products. Improving consumer education would thus be a good opportunity towards increasing purchase of fish from sustainable aquaculture. A well-informed consumer is willing to spend more on sustainable and locally produced seafood with high animal welfare standards (Arru et al., 2019; Birch et al., 2018; Govzman et al., 2021). This increased awareness would consequently allow more products to be sold and encourage farmers to improve their sustainability and fish welfare owing to increasing demand.

Moreover, educational campaigns should be launched on how to prepare fresh fish to make seafood more appealing to the consumers. During many of my semi-structured interviews, participants were found to be unaware on how to prepare fresh fish. Therefore, they purchase products already processed, which are less sustainable than locally aquacultured fish because of transportation and processing procedures (Carlucci et al., 2015). Moreover, a number of other studies support my findings that the knowledge of whole fish preparation is severely limited, particularly in countries without a long fishing tradition (Brunsø et al., 2009; Carlucci et al., 2015; Govzman et al., 2021; Wien et al., 2020). Another possible opportunity would be to use farmed fish in already processed products to simply eliminate this parameter.

Chapter III as well as several other studies have further shown that consumers often feel overwhelmed by the variety of eco-labels (Alfnes et al., 2018; Asche & Bronnmann, 2017). With regard to sustainable consumption, the use of life cycle assessments for aquaculture products appears to be an excellent complement to eco-labels. Adding LCA on the products could help to increase consumers' awareness and restore their trust. A thoughtful use of LCAs could therefore provide consumers with important information about the product. Examples include carbon footprint, impact on the environment, as well as water and resource consumption (Altiok et al., 2021; Cao et al., 2013; Cordella et al., 2020; FAO, 2022). By providing the information and adjusting prices accordingly, consumers could decide for themselves which criteria they prefer when purchasing their food. Unfortunately, in the field of LCA, adequate and reliable data is often difficult to obtain and even more difficult to verify (Madin & Mukaratirwa, 2015). Future campaigns thus need to develop a simple and reproducible verification process that guarantees traceability and accuracy to the consumer. That could shift consumer perceptions and awareness and potentially increase purchases of seafood from sustainable aquaculture.

Simultaneously, alternative feed concepts should be implemented in intensive fish farming. The feed intake is the main factor determining efficiency and cost as well as maximising production efficiency in a fish farm (Assan et al., 2021). As discussed in **Chapter IV**, the sustainability of carnivorous fish feed is important due to high content of fishmeal and fishoil from marine fish stocks (Hua et al., 2019; Luthada-Raswiswi et al., 2021; Owsianiak et al., 2022; Willer et al., 2022). Balancing increasing aquaculture production with sustainable resource management presents a complex challenge.

Nevertheless, in order to ensure human nutrition and achieve the SDGs in the next decade, it must be addressed (Bank et al., 2021; FAO, 2022; Fonseca et al., 2020; Hua et al., 2019). A promising solution is to replace fishmeal from marine fish stocks with hydrolysates from CE. Moreover, depending on the fish species, a significant diet proportion can be substituted with plant- and terrestrial animal-derived proteins, as well as insectmeal. This transfer could preserve resources, foster circular economy, and shift consumer perceptions towards a more positive attitude towards intensive fish farming.

The goal of reducing environmental impacts and improving eco-efficiency by staying within planetary boundaries is a major challenge. Planetary boundaries were first introduced by Rockström et al. (2009) and describe a safe margin of action for humanity based on intrinsic biophysical processes, which regulate the balance of the Earth system (Steffen et al., 2015). Incorporating environmental impacts, such as carbon footprint disclosure on a product, can help raise the awareness of consumers and alter their purchasing patterns (Madin & Mukaratirwa, 2015). For instance, carbon footprint indication could lead to preferred purchasing of local products due to lower carbon emissions.

Looking ahead, we note that younger generations place great emphasis on environmentally friendly food, sustainability, and animal welfare (Kamenidou et al., 2020; Su et al., 2019; Zuo et al., 2022). **Chapter V** presents an approach to adapt turbot circadian rhythms in RAS for optimised aquaculture practices. Modifying feeding regimes in response to peak metabolites, such as BCAAs, sugars or stress metabolites, like cortisol, could help improve fish growth at no additional cost to the farmer. The metabolite levels could indicate the time of the day at which stressful activities such as transport, feeding, or handling should be avoided to improve fish welfare and decrease the risk of disease outbreaks. Metabolism is highly species-specific, and identifying daily variations in metabolites needs to be addressed for each species individually. Turbot has never been researched for its metabolic profile before. My findings record the first trends in its circadian rhythm, where many amino acids as well as sugars peaked at 3 p.m.

Combining novel alternative feed ingredients with optimised feeding and handling regimes could improve profitability and welfare of intensive fish farms. To this end, nuclear magnetic resonance (NMR) spectroscopy provides an accurate tool for metabolic profiling that allows qualification and evaluation of the effects of experimental feeds in the context of fish nutrition (Tavares et al., 2022). The additional display of LCA attributes like animal welfare, carbon footprint, or resource reuse on aquaculture products might benefit consumers by making locally produced fish from intensive aquaculture more attractive than imported finfish. This, in turn, would benefit the environment, local economies, and the welfare of fish, as well as contribute to the achievement of the SDGs.

Limitations and future recommendations

My dissertation focused on consumer analysis and improving intensive fish aquaculture practices, with Section 6.4 identifying potential implications for aquaculture farmers. Nonetheless, limitations exist in the various experiments. Analysing these constraints could contribute to determine the bottlenecks and provide recommendations for the future.

In **Chapter III**, lack of consumer knowledge is cited as the first barrier to supermarket demand for sustainable aquaculture products. However, for this part of my work, only eight knowledge transfer events were attended along with surveys. An additional study should further explore my trend and collect additional supporting data. In this context, the interactive posters could contain more detailed questions to substantiate the findings. The trend was that greater scientific aquaculture knowledge leads to more sustainable purchases. An example for a more detailed question might be the distinction between aquaculture and fishery products. This question was originally in the pretest, but it was removed after I discovered that consumers are often confused about what type of seafood they are purchasing. Again, this finding indicates a lack of consumer knowledge, which needs to be addressed in the future. My work has also demonstrated that attendees at scientific conferences and seminars are more willing to participate in a quick interactive poster survey than an online questionnaire. Nevertheless, providing the right incentive could possibly increase the number of online participants. I would propose an additional online survey in which participants could win a prize if they responded to all the questions. The pretest has to indicate how long it takes to answer the survey carefully, and participants who take too little or too much time would be excluded from the survey. Time is a good indicator of the quality of the questionnaire and could help identify scammers. Participants would also need to self-verify to eliminate completing the questionnaire more than once. Compared to quick poster surveys, online surveys could capture more details about knowledge levels and purchasing behaviour, which might help develop more targeted campaigns.

However, most campaigns in the European Union do not focus either on the generational or national differences. This strategy has to be questioned in order to increase awareness and encourage sustainable purchasing of aquaculture products. A first approach might be to introduce (especially to the younger generation) the “new” production system of the circular economy, including RAS. Research has shown that younger generations are especially concerned with sustainability (carbon footprint) and upcycling of food to avoid food crises and wastes (Kyma¨la¨inen et al., 2021). Hence, the development of new feed concepts (**Chapter IV)** with aquaculture byproducts and locally cultivated plants could change perceptions towards more positive attitudes in aquaculture products.

Another theme that emerged from the poster surveys was between reported and actual product purchases. Most people indicate the importance of animal welfare, sustainability, and regionality on a product. Nevertheless, several studies show that the supermarket’s primary focus is on the price (Gidlöf et al., 2017; Van Herpen & Van Trijp, 2011). Future studies should therefore combine surveys with actual purchase behaviour. To do so, interactive posters could be placed in the entrance area of a supermarket, and survey participants could be interviewed again after their purchase. These results could then be compared with those people who did not participate in the poster survey in the entrance area. This approach could show whether raising awareness at the start of shopping changes purchasing behaviour. Studies suggest that raising awareness and providing additional information encourages people to buy more sustainable products (Menozzi et al., 2020; Van Herpen & Van Trijp, 2011).

I am aware of the fact that these findings are difficult to validate due to biased perceptions of the consumers as well as the categorisation of the scientific knowledge itself. Indeed, personal perspective surveys always contain a factor for error, especially on contested and highly normative topics, such as individual food preferences. To this end, I am aware that my work provides only a first impression on this subject and further detailed research is needed to prove my findings.

Chapter V deals with the circadian rhythm of turbot and demonstrates an attempt to obtain insight into the animals' 24-hour metabolism. Unfortunately, the method applied prevented the recording of the animals' melatonin levels. Melatonin is well-known to be the major metabolite affected by light conditions and diet composition and thus plays a critical role in intensively farmed fish (Acharyya et al., 2022; Gao et al., 2021). Consequently, understanding the daily melatonin expression in turbot could help identify best practices for slaughter, feeding, and handling. Moreover, aquaculture operations could benefit from including melatonin supplements in feed at certain times of the day to reduce stress in the animals. Upcoming studies should concentrate on monitoring melatonin production in turbot on a 24-hour cycle to determine diurnal and nocturnal rhythms. Moreover, the methods used to determine lactate, test stripes and NMR spectroscopy showed some bottlenecks as well. Lactate can be used to assess both stress and immune responses and displayed different values in the two methods used. The test strips gave significant values between 3 a.m. and 9 a.m. At the same time, NMR spectroscopy showed a peak at 3 p.m. Lactate concentrations were often lower than the threshold of the test strips, rendering the results questionable. Blood glucose was also analyzed both by test strips and NMR spectroscopy and displayed similar values and peaks with both methods. I concluded that lactate levels were too low for the test strips, and more studies are needed to reproduce the trend observed in the NMR. Identification of the lowest immunological barrier is critical as it can help optimize feeding, transportation, and handling in RAS, as well as reduce the risk of disease outbreaks in aquaculture operations. This could help improve animal welfare without incurring additional costs to the farmer.

A new area of research is environmental enrichment to improve the welfare of fish in intensive farms (Arechavala-Lopez et al., 2022). Different types of enrichments exist, namely physical (e.g. structures or substrates), sensory (e.g. visual, auditory, or chemical), occupational (e.g. hydrodynamics and play), social, or nutritional (Arechavala-Lopez et al., 2022; Zhang et al., 2022). Such enrichments could contribute to reducing disease and stress, leading to faster growth and higher profits with a high standard of animal welfare. I would recommend applying these structures in a way practical for the farmer and repeat 24 h measurements to conclude if the basal stress levels really decrease. Rearing fish under continuously monitored conditions does not only enhance the quality of the product, but might also affect purchasing behaviour. In recent years, increased attention has been observed on how animals are raised and slaughtered. Former studies have indicated the added value of organic farming by environmental, moral, and health aspects (Patle et al., 2020), which is reflected in consumer choices. Hence, promoting organic development and creating a more natural environment for aquaculture fish combined with alternative feed ingredients and species specific feeding and handling regimes could positively influence perception and therefore purchasing behaviour.

Conclusion

The data presented in this dissertation provide new information on opportunities for improving intensive fish farming in Europe. Fish aquaculture intensification, particularly land-based aquaculture (e.g. RAS), will play a critical role in future food security, as well as towards meeting the SDGs. My work highlights potential solutions that could contribute to improving blue growth in the context of aquaculture in Europe while ensuring sustainability goals, fish conservation, and profitability.

My work examines consumer perceptions and contributes to a better understanding on how scientific knowledge and national differences influence purchasing behaviour. Through a combination of different transdisciplinary approaches, I gained insights and a better understanding of the constraining factors. The primary limiting factor I identified was the lack of consumer awareness in supermarkets, where a negative perception of aquaculture products results in fewer purchases. It is necessary to counteract this decline in demand to increase farm profitability, by encouraging fish farmers to invest more money into sustainable feeds and farming techniques. During my surveys, it also became evident that sustainability, animal welfare, and farming conditions are the main consumer concerns regarding aquaculture products. Such negative perception is generally attributable to a lack of knowledge and could potentially be remedied through targeted national education campaigns. An overarching solution to improve sustainability and provide consumers with more comprehensive information could be to supplement existing labels with a life cycle assessment of products.

Life cycle assessment has shown that feed is the most critical limiting factor for RAS sustainability, and my work supports the previous findings. I have addressed alternative diets for seabass (*Dicentrarchus labrax*) to improve sustainability while ensuring growth and health of the animals. The major ingredient for carnivorous fish in commercial aquaculture diets are fishmeal and fishoil. My work demonstrates that fishmeal from marine fish stocks can be replaced by hydrolysates and aquaculture by-products, as well as plant- and animal-derived proteins, without adverse effects. Moreover, the combination of plant and animal proteins, fish hydrolysates, and insects could positively influence consumer attitudes towards more farmed fish. This shift in feed ingredients, as well as improved consumer perceptions, could create incentives among farmers to act in a more environmentally friendly way and support industrial growth focused on a circular economy.

In addition to sustainability, my work has revealed that animal welfare is particularly important for consumers. Hence, my work presents the initial research dealing with the circadian rhythm of turbot (*Scophthalmus maximus*). Turbot is a highly valuable carnivorous species mainly farmed in RAS and is highly valued by consumers. Circadian rhythms regulate the entire metabolism of the animal and are very species-specific, so extrapolation from one species to another is extremely speculative. My results suggest metabolic peaks in amino acids and sugars at 3 p.m. that can be used to optimise feeding regimes and farming practices. In particular, activities on the farm, such as feeding (digestive stress) or transportation, can affect stress levels and should be avoided during peak levels of stress metabolites in the animal. Determination of day and night rhythms of farmed fish becomes crucial for optimising management practices. Exploiting the variation of natural metabolism could help improve animal welfare and growth through adjusting feeding times at no additional cost to the farmer.

This thesis provides a comprehensive picture of the impact of the different interdisciplinary approaches in European aquaculture, from the consumer to the individual fish. Implementing sustainable and regional ingredients in the diets of carnivorous fish can not only decrease the dependency on raw materials but also positively influence consumer purchase towards aquaculture. Combined with optimised species specific feeding and handling times to improve fish welfare, this thesis provides ideas for novel farm practices that improve both farm profitability and consumer perception.

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Jana and Lisa - there are no words for all the love, encouragement, sushi, mojitos and support you have given me every step of the way. Thank you from the bottom of my heart for seeing who I really am, especially during the times I couldn't. Without you guys, I wouldn't be standing here right now. Thank you for being the sisters I never had.

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Annex

Annex 1. Purchasing criteria poster

Which fish do i eat?!

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Which criteria are important for you when buying fish and seafood?

Please mark with a cross!
(Multiple answers possible)

I buy/prefer ... seafood	fresh	frozen	processed	no
I pay attention to the price	Yes		No	
I eat seafood because of its benefits for health (e.g. Omega 3- fatty acids)	Yes		No	
What means quality of the product for you? (please name)				
I pay attention to certification of seafood (e.g. MSC, ASC, ecolabels)	Yes		No	
I pay attention to the origin (e.g. regionality, FAO fishing areas)	Yes		No	
Animal welfare aspects are important	Yes		No	

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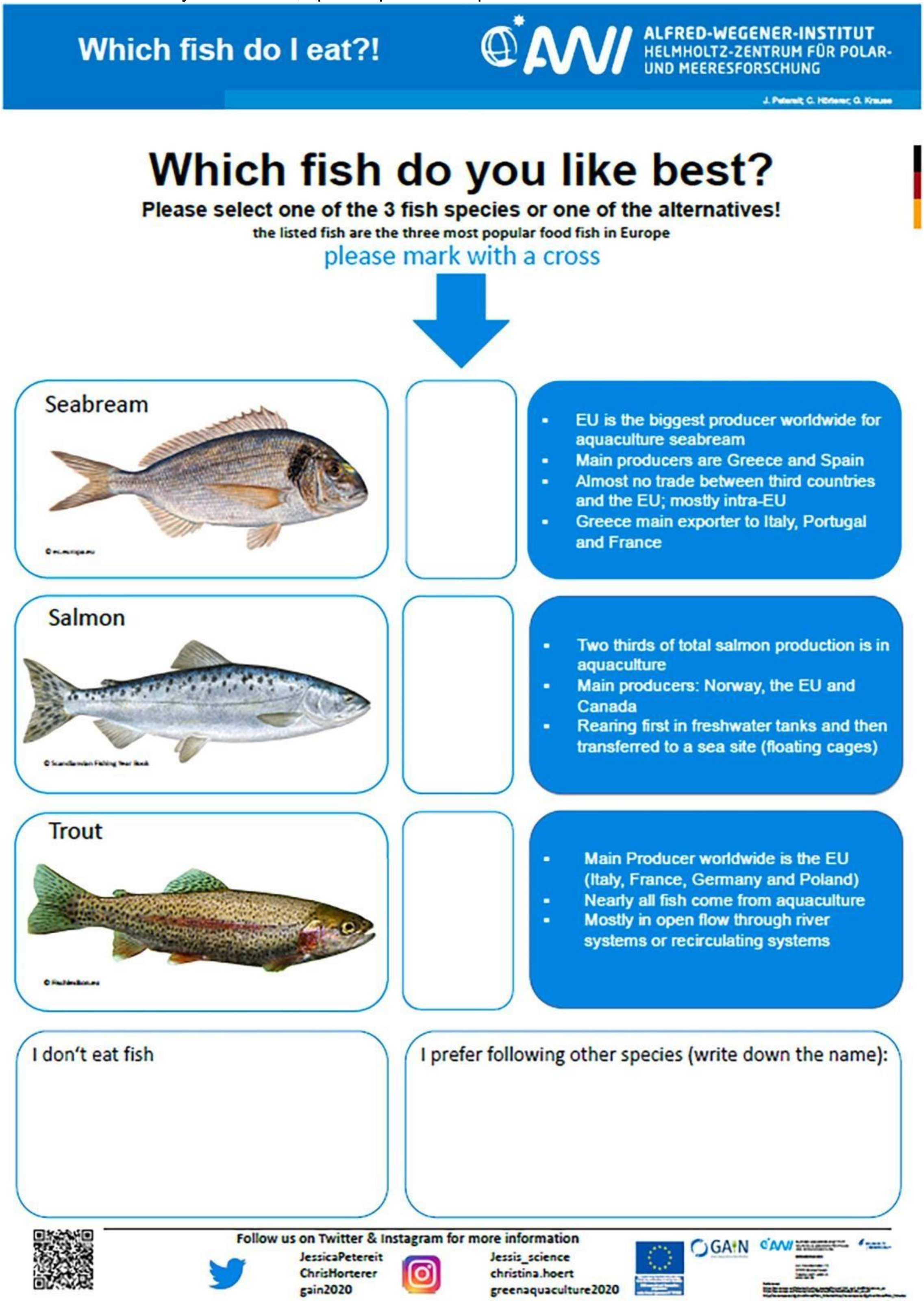
Annex 2. “Which fish do you like best”, species preference poster



Annex 3: Diet formulation of the experimental diets (% dry weight).

FPH: fish protein hydrolysates; I: iodine; Se: selenium; SHP: Salten Havbrukspark AS, partner that produced macroalgae; VPC: vegetable protein concentrate; YM: yeast meal; WU: Wageningen University, partner that produced microalgae.

Ingredients, %	Control	NO PAP	PAP	NO PAP^{+}	PAP^{-}
Fishmeal Super Prime	10.00			15.00	
Fishmeal 60 (by-products)	5.00				
Krill meal				5.00	
Fish protein hydrolysate	3.00				
FPH-trout-head		0.50	0.50	0.50	0.50
FPH-trout-tf		0.50	0.50	0.50	0.50
FPH-turbot-head		0.25	0.25	0.25	0.25
FPH-turbot-tf		0.75	0.75	0.75	0.75
FPH-salmon-head		0.50	0.50	0.50	0.50
FPH-salmon-tf		0.50	0.50	0.50	0.50
Feather meal hydrolysate			5.00		10.00
Porcine blood meal			2.25		5.00
Poultry meal	10.00		14.00		20.00
Insect meal (*Hermetia illucens*)		15.00	10.00	10.00	
Fermentation biomass (*Corynebacterium glutamicum*)		5.00	5.00	2.50	
Fermentation biomass (*Methylococcus capsulatus*)		15.00	10.00	10.00	
Soy protein concentrate	4.40				
Pea protein concentrate		2.50		3.50	
Wheat gluten	6.00	1.50		1.50	
Corn gluten meal	6.00	1.50		1.50	
Soybean meal 48	15.00				
Sunflower meal 40		9.10		6.00	13.60
Wheat meal	11.40	5.70	5.70	5.70	5.70
Whole peas	4.00	11.13	16.36	9.14	14.44
Pea starch (raw)	4.00	4.00	4.00	4.00	4.00
Vit. and Min. Premix—WITH I and Se	1.00				
Vit. and Min. Premix—NO I and Se		1.00	1.00	1.00	1.00
GAIN Macroalgae SHP		2.50	2.50	2.50	2.50
GAIN Macroalgae SHP Se- rich		0.10	0.10	0.10	0.10
GAIN Microalgae WUR Se- rich		0.20	0.20	0.20	0.20
Vitamin E50	0.03	0.03	0.03	0.03	0.03
Betaine HCl	0.10	0.10	0.10	0.10	0.10
Antioxidant	0.25	0.25	0.25	0.25	0.25
Sodium propionate	0.10	0.10	0.10	0.10	0.10
Monoammonium phosphate	1.30	2.65	1.85	1.45	1.60
L-Histidine		0.10			
L-Tryptophan	0.10	0.10	0.10		0.25

Annex

DL-Methionine	0.20	0.40	0.30	0.10	0.30
L-Taurine		0.17	0.09	0.01	0.06
Yttrium oxide	0.02	0.02	0.02	0.02	0.02
Lecithin		0.25	0.25		0.25
Fish oil	5.40	2.70	2.70	2.70	2.70
Salmon oil		9.00	9.00	13.60	9.00
Algae oil		1.00	1.00	1.00	1.00
Rapeseed oil	12.70	5.90	5.10		4.80
total	100.00	100.00	100.00	100.00	100.00

Annex 4: Co-author Paper 1; Informed choice: The role of knowledge in the willingness to consume aquaculture products of different groups in Germany

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Informed choice: The role of knowledge in the willingness to consumeaquaculture products of different groups in Germany

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ABSTRACT

Translating the agricultural eco(logical)-intensification model to European aquaculture hosts the potential for sustainably providing local food for local communities. Using online and printed surveys, we investigated the relationship between social factors such as age, gender, and education to seafood consumption behavior and the perception of aquaculture production. The frequency of seafood consumption was significantly lower in young and female respondents, whereas respondents with a higher level of education consume more frequently. Furthermore, high-frequency seafood consumers had a significant preference for wild-caught fish. Young and female respondents also perceived sustainability of aquaculture lower, whereas the level of education had a significantly positive relation to the attitude towards aquaculture. To foster the acceptance of eco-intensified aquaculture production, we suggest that communication efforts need to be group-tailored, focusing on the reduced environmen- tal impacts, increased animal welfare, and novel products like seaweed to meet the values of the German con-sumer groups.

1. Introduction

Sustainability, defined in the Brundtland (1987) report by the United Nations Commission as the use of resources to meet "the needs of the present without compromising the ability of future generations tomeet their own needs", has become an overarching concept in all as- pects of contemporary human life: ranging from mobility to resource production and consumption. In the light of the climate crisis, younger generations, as seen in the 'Fridays for Future' movement, are reinforc-ing this former call for a stronger balance by asking for more mindful- ness for their future among politicians and the older generations. As part of this sustainability movement, people in developed countries areincreasingly choosing food according to its environmental (e.g. organic, carbon footprint, recyclable packaging), social (e.g. improvement of worker's welfare, access to health services, and school education), and economic (e.g. guaranteed minimum price and access to international markets) sustainability criteria that include aspects of animal welfare and local production (Annunziata and Scarpato, 2014; Lucas et al., 2021). Consumers' attitudes towards sustainable food are often based on personal values, perceived barriers and the confidence of informa- tion received (Corrin and Papadopoulos, 2017; Sanchez-Sabate andSabaté, 2019). Scientists have observed that especially ecology-oriented, female and young consumers are more likely to shift to a meat-reduced, vegetarian, or vegan lifestyle in western countries (Gvion, 2020; Kymalainen et al., 2021; Pribis et al., 2010). However, the effects of sustainability concerns among different consumer groupsin relation to their seafood consumption are rarely studied.

Seafood is often linked to cultural preferences (coastal communitiesvs. land), health beliefs, and consumption habits driven by respective cultural settings (childhood) (Carlucci et al., 2015; Jacobs et al., 2015). Furthermore, it is very diverse in terms of production method (wild vs. farmed) and in relation to the accessible variety of available species groups (finfish, shellfish, algae) (Carlucci et al., 2015; Laborde et al., 2020). Food from the sea contributes 17% to the globally available animal protein and in contrast to fisheries, aquaculture hosts a great potential for sustainable growth (Costello et al., 2020). To achieve this in Europe, where food production is dominated by agriculture, aquaculture production needs to be sustainably boosted, without compromising social and economic benefits while reducing the impact on the environment. This is timely, as for instance from the economic perspective the European Union (EU, 28 member states) has a trade deficit of 33% to date and relies heavily on the import of seafood from non-EU countries (EUMOFA, 2020) that renders the EU vulnerable in terms of marine food security. However, concepts on how to implement sustainable

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growth in aquaculture are rare and criticized for focusing too much on economic growth and not meeting the environmental and social Sustainable Development Goals (SDG) (Cisneros-Montemayor et al., 2021; Farmery et al., 2021). Eco(logical)-intensification, an agricultural model, "to feed the world now and in the future, while maintaining and enhancing ecosystems functions" (Tittonell, 2014). This concept includes different models but basically applies the "harnessing ecosystems services for food security by using e.g. nutrient cycling or biological pest control (Bommarco et al., 2013). Translated to aquaculture it might be a solution for such a sustainable growth of the EU's aquaculture sector. This could mean, e.g. applying circular economy in a farm-to-fork value chain (Maiolo et al., 2021; Schebesta and Candel, 2020) reusing valuable resources such as cuts from fish processing for fish diets (Hoerterer et al., 2022; Vázquez et al., 2019). These sustainable aquaculture products will most likely cost extra for consumers therefore it is necessary to highlight the benefits in audience tailored communication efforts, assuming that consumers can make an informed choice when purchasing seafood. For instance, socio-economic interests, environmental concerns, aesthetic aspects as well as moral, emotional, and personal values all influence the public's acceptance and perception of aquaculture to a different extent (Alexander et al., 2016; Freeman et al., 2012; Mazur and Curtis, 2008; Thomas et al., 2018). Furthermore, the majority of consumers are often uninformed about contemporary aquaculture practices and the benefits of aquaculture products in terms of environment, health, and quality of the products (Bronnmann and Hoffmann, 2018; Feucht and Zander, 2015).

The aim of the study was to identify the socio-demographic factors that influence seafood consumption behavior, the knowledge base on and attitude towards aquaculture of different groups on a showcase basis in Germany. To achieve high relevance and applicability of this study, the authors addressed especially younger age groups (25 years and younger and 26 to 39 years) by placing questionnaires at a conference for young scientists and by a citizen science project with high school students.

2. Material and methods

2.1. Participants

The study was set at two international conferences and in a large online survey addressing the different consumer groups characterized by different age groups and different presumed knowledge about aquaculture. At first, we attended the 'International Conference for YOUNG Marine Researchers' (ICYMARE) 2019 in Bremen which was characterized by participants who were all aged under 40 years (see Table 1). At the Aquaculture Europe Conference AE2019 in Berlin, we conducted a subsample addressing specifically research experts and practitioners from the aquaculture sector with higher average age (26 years and older). At last, we included high school scholars following a citizen science approach under the 'HIGH school of Science and Education at the AWI' (HIGHSEA at the Alfred Wegener Institute for Polar and Marine Research, Bremerhaven) program. The students translated and adapted the existing questionnaire to German used at the ICYMARE and distributed it as an online survey among the public with a lower average age (25 years and younger).

2.2. Questionnaires

The study's methods resemble a set of potential quantitative and qualitative approaches from a social science stance (Kelle, 2014; Levitt et al., 2018). All methods were pre-tested and outcomes of the first surveys were further refined. The foundation for this study were 442 online and printed questionnaires with the same design and questions, which were distributed in English at the ICYMARE and AE2019 conferences and via email in German language by the HIGHSEA scholars following the snowball principle. The questionnaires were used as an explorative survey method to collect self-reported qualitative (Thronicker et al., 2019) and quantitative data within different social groups in a national context by combining predetermined and open-ended questions (Altintzoglou et al., 2017).

Table 1
Socio-demographic characteristics of survey respondents of the HIGHSEA online survey and at the ICYMARE and AE2019 conferences.

Socio-demographic characteristic	Subclassification	Public	Science		Total
		HIGHSEA	ICYMARE	AE2019	
Age	n	331	29	2	362
	25 years and younger	44%	27%	0%	42%
	26–39 years	22%	73%	100%	27%
	40 years and older	34%	0%	0%	31%
Gender	n	326	28	2	356
	Female	50%	89%	100%	53%
	Male	50%	11%	0%	47%
Level of Education	n	322	27	2	351
	School	49%	0%	0%	45%
	Vocational	13%	0%	0%	12%
	Academic	38%	100%	100%	43%
Distance to sea	n	332	26	2	360
	Close (walking distance)	17%	31%	0%	18%
	Relatively close (by car)	65%	58%	100%	64%
	Relatively far	14%	12%	0%	13%
	My country is landlocked	5%	0%	0%	4%

ICYMARE: International Conference for Young Marine Researchers September 24–27 2019 in Bremen; AE2019: Aquaculture conference of the European aquaculture society October 7–10 2019 in Berlin; HIGHSEA: 3-year scholar program of the Alfred Wegener Institute in Bremerhaven.

The first part consisted of five predetermined questions of which three were based on the concept of the 5-point Likert-scale and adapted to the ordinal data collected in this study (Allen and Seaman, 2007) and two based on categorical data which were ranked later. After Almeida et al. (2015), the respondents were asked to indicate their frequency of seafood consumption on a 5-point scale ranging from 1 = "never" to 5 = "at least once a week" and the options "I don't know" and "prefer not to answer". The respondents were asked to self-assess their knowledge about aquaculture production based on a 5-point scale ranging from 1 = "no experience" to 5 = "excellent knowledge" as well as to state their perception of sustainability of fish farming based on a 5-point scale ranging from 1 = "not sustainable" at all to 5 = "very sustainable". Furthermore, the respondents were asked to give their preference in seafood origin in the in the categories "wild", "aquaculture", "unknown", "no preference") and their attitude towards aquaculture by agreeing to positive, neutral and negative statements. The general attitude towards aquaculture was based on specific positive ($n = 3$), negative ($n = 4$), or neutral ($n = 1$) statements the respondents were asked to agree with. The statements addressed social, economic, and environmental aspects of aquaculture practices (see Fig. 1).

Open-ended questions were used to capture the attitude towards aquaculture, as respondents were able to comment on "other", and in addition in the HIGHSEA survey "How do you define sustainability?". To analyze these open-ended questions, we applied a qualitative content analysis (Bryman, 2004), which can be used on digitized survey data, protocols, and interview transcripts that are the output of the semi-structured interviews, focus groups, workshops,and questionnaires.

In the second part, socio-demographic characteristics were collected and evaluated, since we expected that distance to the sea, level of education (Anacleto et al., 2014), gender, and age (NSC, 2019) affect the frequency of seafood consumption as well as knowledge and perception

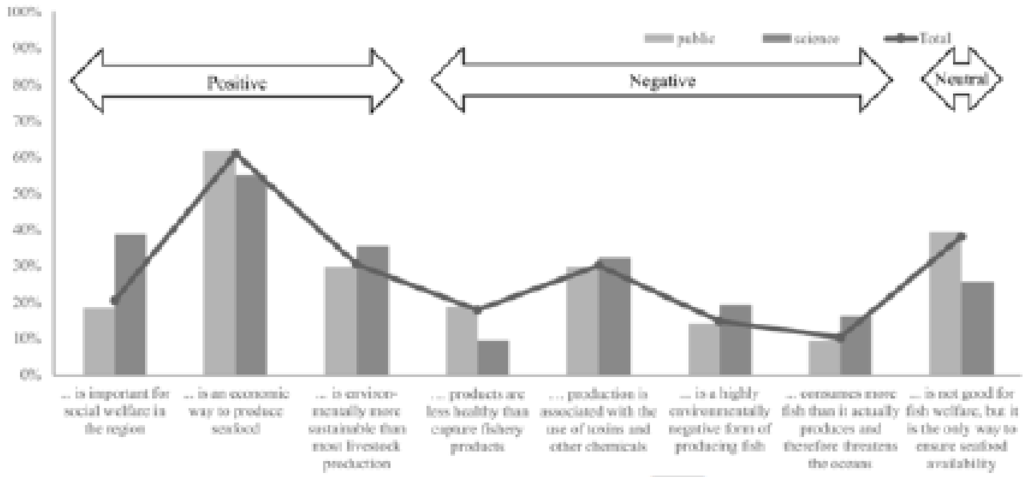

Fig. 1. Percentage of agreement to statements "Aquaculture..." of questionnaire respondents from public (n = 277) and science community (n = 31). Arrows with "positive", "negative" and "neutral" indicate the statements' connotations. Multiple answers possible (n = 724 of 308 answered questionnaires).

of aquaculture. The county of origin was asked in the ICYMARE and AE2019 questionnaires but was excluded from the HIGHSEA questionnaire, due to the German language and distribution range. The level of knowledge is related to the proximity to aquaculture farms (Freeman et al., 2012; Mazur and Curtis, 2008; Thomas et al., 2018) and frequency of seafood consumption (Aarset et al., 2004; Almeida et al., 2015), thus points out to the role of prior exposure (Ladenburg and Krause, 2011).

Overall, the reach and response rate differed strongly between the addressed audiences. At the ICYMARE both printed and online versions of the questionnaire were provided but whereas 46 of 50 printed versions were filled, only six respondents used the online version (N = 52) and 29 respondents stated Germany as their country of origin. At the AE2019 also both versions were provided but due to logistic reasons, we only were able to retrieve the online versions (N = 5), whereas only two respondents stated Germany as their country of origin. The HIGHSEA questionnaires had a high response rate (N = 385). However, 51 questionnaires were incomplete and therefore excluded from the data. The number of analyzed questions differs between questionnaires because some respondents choose not to answer one or two of the demographic characteristics (n = 17).

2.3. Regression and data analysis

A generalized linear regression model with a significance level of $P < 0.050$ was used to test the relationship between continuous response variables and predictors such as consumer demographics (Agresti, 2007). Continuous response variables were defined as preference of the origin of consumed seafood (ranked: 1 = "aquaculture", 0 = "no preference", −1 = "wild", answers with "unknown" were not included), the frequency of seafood consumption (5-point scale: 1 = "never" to 5 = "at least once a week") and the attitude towards aquaculture (ranked: 1 = positive, 0 = neutral or − 1 = negative). The ordinal data on the respondents' self-assessment on knowledge about aquaculture (5-point scale 1 = "no experience" to 5 = "excellent knowledge") and the perception sustainability of aquaculture (5-point scale 1 = "not sustainable" at all to 5 = "very sustainable") in relation to the demographic groups was analyzed with Kruskal-Wallis One Way ANOVA on ranks based on the medians and 25% and 75% percentiles using the Dunn's method for All Pairwise Multiple Comparison Procedures with an overall significance level of $P < 0.050$. The demographic groups were defined as age groups of *25 years and younger*, *26–39 years* and *40 years and older*, gender identification as *female* or *male* and education in *school*, *vocational training*, and *academic*. Furthermore, the questionnaires were categorized by the presumed level of knowledge about aquaculture from low in the *public* (HIGHSEA respondents) to high in the *science* community (ICYMARE and AE2019 respondents). The linear regression model was fitted with all potential predictors. Predictors with no correlations to the response variables were sequentially eliminated from the results based on *p*-values ($P \geq 0.050$). Analysis was conducted using SigmaPlot statistical software (12.5, Free Software Foundation, 2020).

3. Results

We focused on how the socio-demographic factors age, gender, and education (see Table 1) and frequency of seafood consumption affect the knowledge about, perception of, and attitude towards aquaculture in Germany. The age distribution in the study was slightly skewed towards the *25 years and younger* age group (42%), whereas the age groups *26–39 years*, and *40 years and older* represent a similar amount of respondents (27% and 31%, respectively). Further identified as *female* (53%) and had a high level (55%) of education. Due to the geographical focus of this study, in the following results, we present only the data from respondents, who stated Germany as their country of origin.

Respondents from the questionnaire addressing the public answered, "How do you define sustainability?" in 95 of 385 questionnaires and we counted how often keywords were used. 'Resources', 'protect', and 'nature' or related words were mentioned most often and each occurred in 25% of the answers. 'Lasting' and 'intrusion' occurred in 20% and 17% of the answers given by the respondents, respectively. 'Balance', 'food', 'damage' and 'consume' occurred in 9% of the answers. 'Generation', 'production' and 'environment' were used in 7% of the answers. 'Life', 'regeneration' and 'handling' were used in 6% of the answers.

3.1. Seafood consumption behavior, preference and attitude towards aquaculture in relation to age, gender, and education

Overall (N = 385), 60% of respondents consume seafood "at least once a month" (high-frequency). However, the frequency of seafood consumption significantly differs in relation to age, gender, and level of education (see Table 2) and increases with age (linear regression, $t = 7.024$; $P < 0.001$). It is noticeable that 18% of the respondents aged *25 years and younger* stated that they "never" consume seafood and 52% of the respondents aged *40 years and older* consume seafood

Table 2
Frequency of stated seafood consumption in relation to age, gender, and education.

		n	Never	Less than once a year	Less than once a month	At least once a month	At least once a week	P
Total		385	12%	7%	20%	31%	30%	
Age	25 years and younger	152	18%	10%	26%	27%	19%	< 0.001 (S)
	26–39 years	96	8%	11%	22%	40%	19%	
	40 years and older	114	5%	0%	10%	33%	52%	
Gender	Female	166	16%	10%	23%	25%	25%	0.002 (S)
	Male	159	7%	5%	18%	37%	33%	
Education	School	159	16%	10%	23%	25%	25%	< 0.001 (S)
	Vocational	42	7%	10%	17%	45%	21%	
	Academic	150	7%	4%	17%	37%	36%	

n = number of answers given per group; linear regression was used to identify statistical differences with significance level P < 0.050 (S) and P ≥ 0.050 (NS).

"at least once a week". In relation to gender, *female* respondents consume seafood less frequently compared to *males* (linear regression, $t = -3.103$; $P = 0.002$). Similar to age, the frequency of seafood consumption increases with the level of education (linear regression, $t = 4.110$; $P < 0.001$), whereas 16% of respondents with *school* education "never" or rarely consume seafood. Regarding respondents with a higher level of education, more respondents with *academic* background consume seafood at a higher frequency than respondents with *vocational* training.

Interestingly, overall ($N = 364$), only 7% of the respondents prefer "aquaculture" products compared to 31% who prefer "wild" products (see Table 3). However, 37% have "no preference" or it depends on the type of seafood product they buy (e.g. smoked salmon, fish fingers, etc.). One quarter stated that they do not know whether the products they buy are from aquaculture or the wild ("unknown"). Noteworthy, the preference for "aquaculture" or "wild" products was not correlated to age group, gender, or education (linear regression; $P = 0.050$). However, respondents that consume seafood "at least once a month" (high-frequency) have a lower preference for "aquaculture" products and at the same time prefer i.e. "wild" seafood compared to respondents that consume "less than once a month seafood" (low-frequency) (linear regression, $t = -2.537$, $P = 0.012$). Moreover, 64% of low-frequency consumers state not to prefer a certain origin compared to high-frequency consumers (31%).

3.2. *Knowledge and perception of aquaculture production in the public and science community*

A central issue of the questionnaires was placed on capturing the existing knowledge about aquaculture, the perceived sustainability of aquaculture (Table 4), and the plurality of attitudes on aquaculture (Table 5).

Overall, the knowledge about aquaculture from survey participants' self-assessment based on a 5-point scale of 1 ("no experience") to 5 ("excellent knowledge") had a median of 2.0 (1.0–3.0) among all German respondents ($N = 300$), with no differences between the three age groups (One-way ANOVA on ranks, $P = 0.730$) and gender (One-way ANOVA on ranks; $P = 0.136$). Furthermore, the self-assessment of the existing knowledge base significantly increased (One-way ANOVA on ranks, $P < 0.001$) with the level of education from *school* education and *vocational* training (2.0 (1.0–3.0) and 2.0 (1.0–2.25), respectively) to *academic* education (2.0 (2.0–4.0)) and from the *public* (2.0 (1.0–3.0) to the *science* community (4.0 (2.0–4.0)).

The rating of the sustainability of aquaculture among all German respondents had a median of 3.0 (2.0–3.0) ($N = 339$) with significant differences between the age groups and gender. The age group of *40 years and older* ranked aquaculture with a median of 3.0 (2.0–3.0) significantly more sustainable (One-way ANOVA on ranks, $P = 0.033$) than the median of 2.0 (1.0–3.0) in the age groups *25 years and younger* and *26–39 years*. *Male* respondents rank sustainability of aquaculture production with a median of 3.0 (2.0–4.0) significantly higher (One-way ANOVA on ranks, $P < 0.001$) than *female* respondents (2.0 (2.0–3.0)). The level of education and audience (*public*, *science* community) did not affect the sustainability ranking (One-way ANOVA on ranks, $P = 0.918$).

Overall, 52% of respondents ($N = 338$) have a positive attitude towards aquaculture, which was not influenced by age, gender or the audience. However, respondents with a high level of education have a significantly more positive attitude towards aquaculture than those with *school* education (linear regression, $t = 2.414$, $P = 0.016$). However, the overall attitude did not differ among the *public* (HIGHSEA, $n = 277$), and the *science* community ($n = 31$) (linear regression, $t = 0.155$, $P = 0.908$).

Interestingly, 39% of respondents from the *science* community ($n = 31$) agree with the positive statement that 'aquaculture is important for social welfare in the region', whereas only 19% of public respondents ($n = 277$) agree (total 21%). The majority (61%) of the respondents from all groups agree with the positive statement that 'aquaculture is an economic way to produce seafood'. Comparing terrestrial livestock production with aquaculture, 35% of the science community and 30% of the *public* agree with the positive statement that 'aquaculture is more sustainable than terrestrial livestock production'. More public than *science* respondents agree with the negative statement that 'aquaculture products are less healthy than capture fisheries' (19% and

Table 3
Stated preference of production method of seafood in relation to age, gender, education, and frequency of seafood consumption.

		n	Aquaculture	No Preference	Wild	Unknown +	P
Total		364	7%	38%	30%	25%	
Age	25 years and younger	139	5%	35%	27%	34%	0.287 (NS)
	26–39 years	91	9%	44%	25%	22%	
	40 years and older	111	9%	29%	41%	21%	
Gender	Female	175	7%	34%	33%	26%	0.527 (NS)
	Male	161	7%	35%	30%	27%	
Education	School	146	7%	29%	30%	34%	0.243 (NS)
	Vocational	40	8%	44%	31%	17%	
	Academic	144	5%	30%	38%	28%	
Seafood consumption	Less than once a month	119	9%	44%	22%	25%	0.012 (S)
	At least once a month	225	6%	32%	35%	27%	

n = number of answers given per group; linear regression was used to identify statistical differences with significance level P < 0.050 (S) and P ≥ 0.050 (NS); + the category unknown was not included in the linear regression analysis.

Table 4
Self-assessed knowledge base and the perception of the sustainability of aquaculture production.

		Knowledge		Sustainability	
		n	Median	n	Median
Total		300	2.0 (1.0–3.0)	339	3.0 (2.0–3.0)
Age	25 years and younger	125	2.0 (1.0–3.0)	146	2.0 (2.0–3.0)a
	26–39 years	81	2.0 (1.0–3.0)	90	2.0 (2.0–3.0)ab
	40 years and older	92	2.0 (1.0–3.0)	101	3.0 (2.0–3.0)b*
Gender	Female	162	2.0 (1.0–3.0)	174	2.0 (2.0–3.0)a
	Male	134	2.0 (1.0–3.0)	157	3.0 (2.0–4.0)b**
Education	School	129	2.0 (1.0–3.0)a	149	2.0 (2.0–3.0)
	Vocational	30	2.0 (1.0–2.25)a	36	3.0 (2.0–3.0)
	Academic	131	2.0 (2.0–4.0)b**	143	3.0 (2.0–3.0)
Audience	Public	269	2.0 (1.0–3.0)a	311	3.0 (2.0–3.0)
	Science	31	4.0 (2.0–4.0)b**	28	2.5 (2.0–3.0)

n = number of answers given per group; Kruskal-Wallis One-way ANOVA on ranks, values given as medians and the 25% and 75% percentiles, values with different letters within the same columns of one group are significantly different (Dunn's method, $P \geq 0.050$), *$P < 0.050$; ** $P < 0.001$.

Table 5
Attitude towards aquaculture in percentage.

		Attitude				
		n	positive	neutral	negative	P
Total		338	52%	22%	26%	
Age	25 years and younger	124	52%	19%	29%	0.650 (NS)
	26–39 years	81	53%	30%	17%	
	40 years and older	103	56%	15%	29%	
Gender	Female	151	50%	24%	26%	0.487 (NS)
	Male	151	58%	17%	26%	
Education	School	132	48%	20%	33%	0.016 (S)
	Vocational	32	56%	25%	19%	
	Academic	135	60%	20%	20%	
Audience	Public	277	53%	20%	26%	0.908 (NS)
	Science	31	52%	25%	23%	

n = number of answers given per group; linear regression was used to identify statistical differences with significance level $P < 0.050$ (S) and $P > 0.050$ (NS).

Table 6
Categorized comments on the option "other" in the question about the attitude towards aquaculture.

Category	Statement
(1) The sustainability of aquaculture depends on the culture system (IMTA, RAS, and intensity), cultured species and regionality.	*"Aquaculture, if done in a multi-trophic and local scale can be a very sustainable alternative for seafood"*
	"for some species already very sustainable and good; but improvements needed for other species"
	"It all depends on the methods/type of aquaculture"
	"There are semi-intensive AQ systems. AQ can be a sustainable way for fish production, more research and improvement of nutrition, animal welfare has to be done"
	"[...] I think it depends on the manner in which it is done. [...]"
	"Aquaculture is a diverse field; I prefer some production methods to others."
	"Aquacultures are only ecological reasonable as organic aquacultures"
(2) Aquaculture is necessary to ensure food security.	*"Aquaculture can be necessary to other regions"*
	"it's a necessity"
	"Aquaculture if done right can be beneficial to feeding humans. [...]"
	"The main point is that the fish price and the quality is right"
	"Aquaculture is a useful addition to traditional fishing)"
(3) Aquaculture is not sustainable because of pollution by antibiotics and the spread of parasites, impacts on wild populations.	*"negative effects due to use of antibiotics and spreading of diseases and parasites"*
	"they use antibiotics in aquaculture and thus pollutes the ocean"
	"spread of parasites, farmed fish are fed fish"
	"[...] If toxins, overpopulation, wrong waste management occurs, aquaculture can be detrimental to the environment"
	"Aquaculture must be ecologically compatible, otherwise it damages and threatens wild fish, for example, salmon in western Canada"
(4) Respondents are uninformed	"There is too little information on the subject."
	"No knowledge available"

10% respectively). In contrast, more respondents from the *science* community agree with the negative statements that 'aquaculture is a highly environmentally negative form of producing fish' (19% and 14% respectively) and 'aquaculture consumes more fish than it actually produces and therefore threatens the oceans' (16% and 10% respectively). Approximately one-third (30%) of all respondents agree with the negative statement that 'aquaculture production is associated with the use of toxins and chemicals'. *Public* respondents agree in 40% of the answers with the statement that 'aquaculture is not good for fish welfare, but it is the only way to ensure seafood availability', whereas fewer *science* respondents agree with this statement (26%).

In the option "other", the respondents were able to give their statement, which 29% of the *science* community and 7% of *public* respondents did. The answers given in the option "other" could be grouped into four categories (see Table 6). Categories (1) 'The sustainability of aquaculture depends on the culture system (IMTA, RAS, intensity), cultured species and regionality.' and (3) 'Aquaculture is not sustainable because of pollution by antibiotics and the spread of parasites, impacts on wild populations.' are centrally addressing environmental issues. In contrast, category (2) 'Aquaculture is necessary to ensure food security.' addresses primarily societal and economic issues. Only a few respondents in the *public* survey stated that they (4) '[...] are uninformed'. Respondents mentioned that aquaculture "can be sustainable if..." or "some aquaculture practices are sustainable, others need to be improved..." showing that both, positive and negative attitudes of aquaculture are centrally correlated to the production method, scale, and environment (Category 1). The respondents are also aware that aquaculture is important for food security (Category 2) "...if done right..." and "necessary to other regions". The *public*, as represented by the HIGHSEA survey, displays an overall more negative attitude and agrees more with the negative aspects, i.e., focusing on the negative environmental impacts, the use of toxins and chemicals associated with aquaculture production, pollution and the threat to wild populations (Category 3).

4. Discussion

The questionnaire was developed to look in more detail at various aspects that relate to the social acceptance of aquaculture in Germany. In addition, these questionnaires also enquired about the common understanding of sustainability to achieve a better understanding, of what consumers and stakeholders assume what sustainability should entail. The central focus was placed on younger generations (70% of respondents were under the age of 40 years) and their levels of acceptance, which does not represent the age group distribution in Germany as a whole, where 43% contribute to the under the age of 40 years groups and 57% to the 40 years and older group (DESTATIS, 2021). With these results, we derived recommendations on how to potentially improve and tailor information availability for fostering acceptance of eco-intensification measures of aquaculture. As relevant social factors, we identified age group, gender, and educational level that are discussed in more detail according to their influence on the response parameters below. Moreover, we discuss the implications of the subsample in relation to the direct influence of increased knowledge on the change of attitude towards aquaculture.

Influence of social factors on seafood consumption behavior

There appears to be a discrepancy between the voiced preference forwild fish and the actual higher consumption rate of farmed fish that somewhat mirrors the findings of López-Mas et al. (2021). Indeed, our results showed that preference for seafood of a certain production method (wild vs. farmed) was not influenced by age, gender, or educa- tion, but rather by the frequency of seafood consumption. High- frequency seafood consumers (respondents that consume seafood morethan once a month) prefer wild seafood, while low-frequency seafood consumers (respondents that consume less than once a month) are more likely to have no preference. However, as seen in this study, Germans consume less frequently seafood (65% at least once a month) than the average European (70%), but expose the same preference for wild (31%) and aquaculture (9%) products (Eurobarometer, 2018). The younger age group of under 40 years (born after 1980) stated to con- sume seafood less frequently than the 40 years and older group, which is in contrast with the NSC (2019) report that stated that the fish con- sumption was higher in the younger age groups. By large, female re- spondents show similar preferences as the younger age groups, consum-ing less seafood. Furthermore, respondents with a high level of educa- tion are more likely to eat seafood at least once a month. This can be ex-plained by seafood usually being associated with a healthy lifestyle and especially more educated and older aged people have a better under- standing of the health benefits of certain products (Bjørndal et al., 2014), which leads in turn to a higher seafood consumption rate (Heueret al., 2015).

The result that younger and female respondents consume less seafood might be related to the increased awareness of environmental issues of food production and the modern lifestyle of Europeans (Kymalainen et al., 2021; Verbeke et al., 2007b). Several young and noteworthy especially female respondents stated that they do not con- sume seafood at all, which reflects the outcomes of the NSC (2019) re- port and the global trend of meat reduction due to moral and environ- mental reasons (Gvion, 2020; Koch et al., 2019; Pribis et al., 2010). In contrast to this observable trend among young age groups, it is note- worthy that especially in the older and more educated consumers, pos- sible health benefits, taste, and consumption habits might be an under-lying motivation for a prevailing high seafood consumption (Cantillo etal., 2021; Carlucci et al., 2015; Eurobarometer, 2018).

4.1 Perception of sustainability and the attitude towards aquaculture

In order to communicate the benefits of seafood produced in eco-intensified aquaculture production, we need to understand how the different consumer groups perceive and interpret sustainability and the positive and negative dimensions of aquaculture production. Scientists are much more aware of the tradeoffs between the benefits and costs in the ecological, social, and economic dimensions of aquaculture production than the public (Bacher et al., 2014; Chu et al., 2010). The current prevailing societal narrative of aquaculture to date focuses more on the environmental dimensions of sustainability and to a much lesser extent on the social and economic domains (Freeman et al., 2012). The diversity of responses in this study showed that not only academic but also all social groups within a society (e.g. politicians, decision-makers, or- dinary citizens, children, etc.) need a better (common) understanding of sustainability. 'Resources' and 'nature' were most often mentioned as central definitions for sustainability, and surprisingly little attentionwas voiced on social (and economic) factors, rather only related to 'generation', 'food' and 'impact'. In this regard, science is expected to support and become involved in processes of social learning to comply with these new demands (Siebenhüner, 2004). However, the concept of sustainability, its dimensions, and its definition is complex and often viewed one-sided by different stakeholder groups (Béné et al., 2019; Lawley et al., 2019; Risius et al., 2017). For instance, economic stakeholders often focus on economic and environmental sustainability whilst neglecting the social dimension (Hoerterer et al., 2020). Similar to the findings of Lawley et al. (2019), the assumed greater involvement in the topic of seafood production of the scientific community was posi-tively related to the ranking of sustainability.

This somewhat persistent narrow perception of sustainability in thepublic is reflected in the respondents' agreement with aquaculture statements. Public and science respondents alike mainly voiced nega- tive environmental concerns such as the degree of pollution of the ma- rine environment, use of antibiotics and other chemicals. This coincides with other studies, where environmental risks and impacts are noted tobe a major concern and act as an ethical and moral barrier for consump-tion of aquaculture products (Bacher et al., 2014; Bergleiter and Meisch, 2015; Chu et al., 2010; Feucht and Zander, 2015; Mazur and Curtis, 2008). As shown in this study, the public is not as aware of social benefits of aquaculture such as social welfare (see Fig. 1) and food secu- rity (see Table 6) as the informed groups of scientists (Bacher et al., 2014; Krause et al., 2020; Schlag and Ystgaard, 2013). In Whitmarsh and Wattage (2006) the public perceived minimizing environmental damage as the most important objective in the salmon farming indus- try, whereas maintaining employment, improving product quality, avoiding conflicts with other resource users, and ensuring fair prices were perceived as less important with very little variations between the surveyed areas. Indeed, Aarset et al. (2004), Verbeke, et al. (2007b), and Feucht and Zander (2015) showed that there is a perception-realitygap between actual environmental impacts of aquaculture production and the health benefits and nutritional value of aquaculture products, rendering attitude towards aquaculture products more negative, espe- cially fish.

In this study, the public respondents from Germany stressed the im- portance of health issues ("wild-caught fish is healthier than aquacul- ture fish") and animal welfare as well as the price for the product. This links to the findings across Europe that health benefits and higher ani- mal welfare standards are a central driver for seafood purchase and consumption, but often negatively associated with aquaculture prod- ucts (Cantillo et al., 2021; Carlucci et al., 2015; Feucht and Zander, 2014; Rickertsen et al., 2017). Concerning the price of seafood, previ- ous studies have shown that high prices can be a barrier to seafood con- sumption (Carlucci et al., 2015). However, consumers of southern countries such as Portugal appear to be more willing to pay for sustainable salmon, compared to consumers from Norway (Misund et al.,2020). In contrast, German consumers are less willing to pay more for sustainable products or will not purchase a product if the price is higher (Bronnmann and Hoffmann, 2018). However, improved information about animal welfare (Stubbe Solgaard and Yang, 2011), local, domes- tic, or European production (Zander and Feucht, 2017), or 'natural' production methods (Risius et al., 2017), such as pond aquaculture could increase the willingness to pay extra for sustainable aquaculture products. Despite that the younger age groups and female respondents from the public audience ranked the sustainability of aquaculture as low, the attitude towards aquaculture was overall positive (>50%). This is in contrast to previous studies where consumers from different countries and backgrounds had a more negative attitude towards aquaculture and aquaculture products (Rickertsen et al., 2017; Verbeke et al., 2007a). Respondents with higher education or science background have aneven more positive attitude towards aquaculture. This suggests that a higher level of knowledge might lead to a positive attitude towards aquaculture, but its ripple effects on sustainable consumption behavior are not clear (Almeida et al., 2015; Feucht and Zander, 2015; Richter and Klockner, 2017).

In summary, the public needs improved knowledge on aquaculture production and the interwoven plurality of sustainability dimensions therein to order to understand the manifold processes that take place and how these are embedded in our economies, environment, and soci-eties. Such systemic worldviews offer scope towards transformative pathways of future marine food production across Europe. In its wake, forming linkages between different mindsets, worldviews, cultural be- lief systems of sustainability create both conceptual and cultural chal- lenges.

4.2. Does information lead to informed choice?

More often, consumers are rather driven by moral and ethical rea- sons in their seafood purchasing and consumption behavior, such as values and (culturally rooted) daily habits, than by scientific reasoning that acknowledges environmental, social, and economic benefits of lo- cal, domestic or European aquaculture (Schlag and Ystgaard, 2013). That said it is crucial to know how and in what ways improved scien- tific knowledge affects seafood-purchasing decisions. This will allow tailoring better communication pathways to inform about the benefits of eco-intensified aquaculture products that are based on scientific find- ings as well as endorsing the respective consumer's values, culture, andhabits.

In this study, the majority (77%) of the public respondents self- assessed to have a low level of knowledge about aquaculture, whereas the knowledge of the science community respondents was higher. Fur- thermore, the level of education can be positively correlated to the knowledge about aquaculture, which might be related to a higher gen- eral level of knowledge including knowledge about aquaculture. How- ever, some public respondents voiced that they are uninformed and arenot able to agree with the statements about aquaculture. Pretesting showed that the degree of knowledge about aquaculture did not affect the perception of aquaculture. In contrast, previous studies showed that the level of knowledge is related to the proximity to aquaculture farms (Freeman et al., 2012; Mazur and Curtis, 2008; Thomas et al., 2018) and the frequency of seafood consumption (Aarset et al., 2004).

In the exploratory survey at the ICYMARE aquaculture session, three respondents changed their attitude more positive due to improved knowledge about sustainable aquaculture, while the other eight respon- dents did not change their attitude. No one changed the perception to- wards more negative, suggesting that improved information about the sustainability of aquaculture practices and its products could only have positive effects on the perception. However, previous studies have shown that information and improved knowledge could also lead to a shift in consumers' decisions against aquaculture products (Claret et al., 2016; Feucht and Zander, 2015). Due to the small number of respon- dents, the results offer only on a very exploratory scale that there are potential shifts possible in the perceived impacts of aquaculture. These exploratory results indicate that more research is warranted to fully un-derstand the role of improved scientific information in everyday deci- sion-making of food consumption. However, the engagement with trustworthy knowledge holders (scientists presenting aquaculture- related research results) led to a topical perception shift, indicating a learning process on the individual level.

4.3. Implications for a future acceptance of aquaculture products from the eco-intensification approach in Germany

The premise of this study was that social change towards acceptanceof eco-intensification measures in aquaculture would benefit from a better understanding of sustainability thinking among ordinary citizens and especially younger age groups. It is not sufficient for only experts to be knowledgeable about eco-intensification measures in aquaculture. Research insights need to be tailored to the specific needs of the respec- tive audiences in order to develop relevant or meaningful outputs (Krause and Schupp, 2019). What constitutes relevance or meaningful-ness is part of an ongoing negotiation process between academia and society and may vary widely for different social groups and contexts, and different scientific disciplines alike (Hornidge, 2014). For contextu- alization of research findings towards the social realities of stakehold- ers, the requirements of actors from scientific and societal realms need to be understood in order to design a targeted output (Regeer and Bunders, 2003).

In the case of communicating the benefits of eco-intensified aqua- culture production, this study's results conform to previous studies (Risius et al., 2017; Schlag and Ystgaard, 2013; Zander and Feucht, 2017). Tailored communication per consumer group should highlight research insights on new developments reducing environmental im- pacts, animal welfare, and nutritional and health benefits of locally pro- duced seafood products addressing values and habits of the respective groups. In the German context, it might be crucial to communicate the benefits of the application of circular economy in the production of feeds for Europe's most popular fish species like trout (Maiolo et al., 2021), salmon (Vázquez et al., 2019), sea bream and sea bass and the technological advancement for monitoring environmental interaction (Burke et al., 2021; O'Donncha and Grant, 2019). Indeed, the current pandemic and the recognition of how vulnerable globalized food sys- tems are has acted as an accelerator for regional, circular economy thinking (Kaiser et al., 2021). However, communication alone will not be sufficient, since consumers want to rely on the aquaculture industry to follow sustainable standards (Banovic et al., 2019; Feucht and Zander, 2015), produce reliable labeling (Carlucci et al., 2015; Risius et al., 2017), without giving too complex information (Bronnmann and Hoffmann, 2018; Cantillo et al., 2021; Reinders et al., 2016).

It is noteworthy that this study revealed that especially the younger age groups consume less frequently or no seafood than the older groups. This reduction might be mainly due to moral and ethical rea- sons (Verbeke, et al., 2007b), and emphasizing benefits of eco- intensifications measures for animal welfare, no pollution, and absenceof drugs and hormones as well as sustainable fish feed might be crucial for communication for this respective age group (Schlag and Ystgaard, 2013; Zander and Feucht, 2017). Aquaculture advocates, belonging mostly to the older age groups, should leave preconceived notions suchas assumed positive consumer behavior changes if messaging health benefits of seafood consumption (Jacobs et al., 2015), but rather uptake young and critical consumers' interests that revolve more strongly around vegetarian or vegan lifestyle. Scherer and Holm (2020) pro- posed that advocating eating lower trophic levels of seafood might tap into the potential of locally produced marine resources, which acknowl- edges the raising demand for regionalization of food production. In or- der to accommodate the trend of a plant-based diet among the “con- sumers of tomorrow”, aquaculture advocates should promote the pro- duction and consumption of novel plant/algae based aquaculture prod- ucts, such as seaweed. At the ICYMARE aquaculture session some re- spondents stated that the sea grapes (*Caulerpa lentillifera*) presented by Stuthmann et al. (2019) were interesting to them as a novel food. Pro- duction of seaweed is in many ways considered sustainable by not using fished resources as finfish production, as its reputation as a functional food, and its potential for ecosystem services (Buchholz et al., 2012; Garcia-Poza et al., 2020).

5. Conclusion

The presented findings mirror previous studies, in which age, educa-tion, and location of stakeholders influenced the preferences towards a more sustainable lifestyle (Black and Cherrier, 2010; Kapferer and Michaut-Denizeau, 2019; Schoolman et al., 2014) and the willingness to accept higher prices of sustainable products (De Pelsmacker et al., 2005; Stubbe Solgaard and Yang, 2011).

However, the results of this and previous studies do not clearly indi-cate that consumers will choose a more sustainable product based on provided information on the benefits of aquaculture products from eco-intensified production. Even though consumers state that sustainabilityis important for them, their purchase behavior is often run along by val-ues, habits, lifestyle, convenience, and trust in information sources andnot (solely) by scientific reasoning (Carlucci et al., 2015; Feucht and Zander, 2015; Gaviglio and Demartini, 2009; Jacobs et al., 2015). In- stead of relying only on a bottom-up transformation through con- sumers' decision to purchase and consume sustainable aquacultureproducts, the aquaculture industry should also intrinsically aim for a successful transformation to an eco-intensified European aquaculture sector (Almeida et al., 2015; Bergleiter and Meisch, 2015; Lawley et al., 2019; Richter and Klockner, 2017). This might enhance the trust of the consumers in sustainable and especially environmentally friendly pro-duction of food from the seas.

Overall, more factors have to be considered when the aquaculture industry wants to boost sustainable production in Europe. Current and unforeseen developments such as the COVID-19 pandemic host the po-tential to change environmental awareness, sustainable consumption, and social responsibility (Kaiser et al., 2021; Severo et al., 2021).

Furthermore, the aspiration for economic growth and increased con-sumption should be seen more critically, especially in the light of the younger generations having other values than the older generations. Wanting to produce more to sell more, might be the wrong strategy fac-ing lower seafood consumption rates among the younger age group now and in the future. Initiatives like the Blue Growth Agenda launchedby the EU are very important. However, these risk delivering only a part of the promise as they focus strongly on economic dimensions but over-looking other aspects necessary for sustainable seafood production (Eikeset et al., 2018). Scientists (see Ertör and Hadjimichael, 2019) andorganizations such as the High Level Panel for a Sustainable Ocean Economy (HLP or the Ocean Panel), which was created in 2018 advo- cates blue degrowth in order to reduce environmental impacts, securinga future worth living for generations to come.

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Ethics statement

This study was in accordance with the regulations of the German Re-search Foundation (DFG) and the Council for Social and Economic Data (RatSWD), as all collected information was anonymous and non-sensitive and participants are not identifiable. Participants were explic-itly informed about and consented to the aim of the study, the method-ology and about what data will be collected, processed, stored and pub-lished. All data were collected, stored and processes in compliance with the General Data Protection Regulation (GDPR).

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Brundtland, 1987

CRediT authorship contribution statement

Christina Hoerterer: Conceptualization, Methodology, Valida- tion, Formal analysis, Investigation, Data curation, Writing – origi- nal draft, Writing – review & editing, Visualization. **Jessica Pe- tereit:** Conceptualization, Methodology, Validation, Investiga- tion, Writing – review & editing. **Gesche Krause:** Conceptualiza- tion, Methodology, Validation, Writing – review & editing, Supervi- sion, Project administration, Funding acquisition.

Declaration of Competing Interest

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

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Annex 5: Co-author Paper 2 Sustainable fish feeds: potential of emerging protein sources in diets for juvenile turbot (Scophthalmus maximus) in RAS
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Sustainable fish feeds: potential of emerging protein sources in diets for juvenile turbot (*Scophthalmus maximus*) in RAS

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Abstract
In Europe, turbot aquaculture has a high potential for sustainable production, but the low tolerance to fishmeal replacement in the diet represents a big issue. Therefore, this study investigated the effects of more sustainable feed formulations on growth and feed perfor- mance, as well as nutritional status of juvenile turbot in recirculating aquaculture systems. In a 16-week feeding trial with 20 g juvenile turbot, one control diet containing traditional fishmeal, fish oil and soy products and two experimental diets where 20% of the fishmeal was replaced either with processed animal proteins (PAP) or with terrestrial plant pro- teins (PLANT) were tested. Irrespective of diets, growth performance was similar between groups, whereas the feed performance was significantly reduced in fish of the PAP group compared to the control. Comparing growth, feed utilisation and biochemical parameters, the results indicate that the fish fed on PAP diet had the lowest performance. Fish fed the PLANT diet had similar feed utilisation compared to the control, whereas parameters of the nutritional status, such as condition factor, hepato-somatic index and glycogen con- tent showed reduced levels after 16 weeks. These effects in biochemical parameters are within the physiological range and therefore not the cause of negative performance. Since growth was unaffected, the lower feed performance of fish that were fed the PAP formula- tion might be balanced by the cost efficient formulation in comparison to the commercial and the PLANT formulations. Present study highlights the suitability of alternative food formulation for farmed fish.

Keywords Insect meal · By-products · Energy reserves · Mineral trace elements · Circular economy

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Aquaculture has the potential to ensure a reliable supply of seafood for the globally increasing demands and sustainable growth. In order to conserve and sustainably use aquatic resources, the reduction of the environmental footprint of aquaculture practices has become a high priority for the scientific community, producers and consumers. One of the major concerns is the challenge to feed farmed fish with diets that are nutritious but at the same time economically and environmentally sustainable (Glencross et al. 2020). In the last decades, research efforts focused on the identification of major nutritional requirements for important farmed fish such as trout, salmon, sea bass or seabream (FAO 2020; Naylor et al. 2021). These efforts set the foundation for substitution of fishmeal and fish oil originating from wild pelagic fish with other sources (Hardy and Barrows 2003). This resulted in diet formulations with reduced fish content that improve growth and feed performance (Olsen and Hasan 2012).

However, in many carnivorous farmed fish, a total replacement of fish products in the diets is still not feasible. In order to reduce dependence on traditional fishmeal and fish oil, the use of fishery and aquaculture by-products is a good alternative for sustainable aqua- feeds (Bendiksen et al. 2011; Forster et al. 2005; Hua et al. 2019; Whiteman and Gatlin 2005). Processed raw materials such as hydrolysates are more energy efficient than fish- meal from by-products and were shown to improve growth and feed performance in farmed fish (Siddik et al. 2020). Terrestrial plant materials are commonly integrated into commercial fish diets (Abdel- Latif et al. 2022; Gatlin et al. 2007; Naylor et al. 2021; Tacon et al. 2011) enabling even fish-free diet formulations for carnivorous fish. In the case of soybean, however, these products are often associated with unsustainable production, long transportation and a high proportion of genetically modified strains. Furthermore, soybean meals and other vegetable ingredients introduce anti-nutrients, giving rise to a number of problems for the fish such as enteritis and reduced nutrient uptake and bioavailability (Baever ord and Krogdahl 1996; Kaushik et al. 1995; Storebakken et al. 1998). This can be offset—at least to a certain point—by refining the plant protein sources (Glencross 2016; Jia et al. 2022; Naylor et al. 2009; Refstie et al. 2005), which, however, introduces costs and results in a trade-off between fish welfare/health and feed cost. Other alternative protein and oil crops such as pea, rapeseed and lupines proved to be suitable for fish feeds (Burel et al. 2000b; Glencross et al. 2011; Omnes et al. 2015; Øverland et al. 2009; Zhang et al. 2012). However, the availability at a competitive price and regular supply in sufficient quality is still a major issue that needs to be solved (Bähr et al. 2014; Glencross et al. 2020). Moreover, many consumers question whether plant materials are an acceptable and appropriate feed ingredient for carnivorous fish (Feucht and Zander 2015).

Plant material as a basic commodity is used in a wide range of human consumption, feed for terrestrial livestock, biofuels and many other industrial applications. Therefore, the competition is high, and aquaculture feed producers should avoid to totally rely on plant materials. Therefore, researchers and feed producers emphasise that a broader range of alternatives is needed to facilitate the predicted increase in fed aquaculture pro- duction (FAO 2020; Matos et al. 2017). Since the European crisis of the mad cow disease in 1990, terrestrial animal proteins were mostly banned from farmed animal feed formulations. Therefore, research on PAPs in fi feeds is scarce until recently. However, recent studies show that PAPs are suitable alternatives to fish in fish diets (Campos et al. 2017; Karapanagiotidis et al. 2019; Lu et al. 2015; Wang et al. 2015; Wu et al. 2018). However, in 2013 non-ruminant PAPs (processed animal proteins) were re-authorised in the EU under very specific regulations allowing correctly categorised PAP in aquafeeds. The availability in large amounts in the EU and elsewhere as a by-product from food production and its nutritional value qualify PAPs as a sustainable feed ingredient for fish (Tacon et al. 2011).

Recently authorised as novel food and feed in the EU, insect derived products, such as protein and lipids, are valuable ingredients for aquaculture feeds. Insects can valorise unused plant material, not suitable for human consumption, and transform it into valuable nutrients (Newton et al. 2005; Van Huis 2013). They are also part of the natural diet of many freshwater and marine fish species (Henry et al. 2015). Meals derived from the black soldier fly (Hermetia illucens) or mealworm (Tenebrio molitor) were already successfully tested in fish diets for carnivorous fish species such as Atlantic salmon (Salmo salar) (Li et al. 2020), rainbow trout (Oncorhynchus mykiss) (Jozefiak et al. 2019; Rema et al. 2019; Stadtlander et al. 2017) and red seabream (Pagrus major) (Ido et al. 2019).

Other feed ingredients, such as micro- and macroalgae and microbial meals, are emerging as suitable protein and lipid sources for aquafeeds. Microbial biomass, which is produced as a by-product from food, beer and biogas production, can be a valuable ingredient in aquafeeds (Aas et al. 2006; Bendiksen et al. 2011; Oliva-Teles and Goncalves 2001; Olsen and Hasan 2012; San Martin et al. 2020; Tacon et al. 2011). In particular, microalgae are a valuable source with essential fatty acids in diets with a low level of fish oil. Additionally, algae and yeast can act as functional ingredients, increasing the health of farmed fish and crustaceans (Dineshbabu et al. 2019; Refstie et al. 2010; Vallejos-Vidal et al. 2016; Wan et al. 2019).

Novel feed formulations with a broad spectrum of ingredients can balance the ingredients' quality, cost and availability, but most importantly, they need to satisfy the nutritional requirements of the farmed species. Thereby the effects of integrating alternative feed ingredients on fish performance and nutritional status have to be validated. In comparison to fishmeal, alternative ingredients differ in nutritional composition, digestibility of nutrients and availability of minerals (Glencross 2016; Sugiura et al. 1998). This may affect growth, nutrient utilisation and whole body composition of carnivorous fish and lead to an altered energy metabolism and energy allocation. Plant-based and carbohydrate-rich diets influenced the energy reserves, such as the hepatic content of glycogen and lipid in Atlantic salmon, rainbow trout (Krogdahl et al. 2004), Gilthead seabream (*Sparus aurata*) (Robaina et al. 1995) and turbot (*Scophthalmus maximus*) (Miao et al. 2016). Furthermore, plant- based diets affected the mineral composition and availability in rainbow trout (Antony Jesu Prabhu et al. 2018; Read et al. 2014) and Atlantic salmon (Silva et al. 2019; Storebakken et al. 2000).

Turbot is an important species in EU aquaculture due to its high value and reputation and low competition with fishery production (EUMOFA 2018). It has a high potential for sustainable production due to the controlled farming cycle, production practices (RAS and flow-through systems) and its robustness, enabling high-density farming and domestication (FAO, 2005, 2005; Aksungur et al. 2007; Bischoff et al. 2018; EUMOFA 2018; Li et al. 2013). However, as a carnivore, turbot has a low tolerance to fishmeal reduction (Burel et al. 2000a, 2000b; Nagel et al. 2012; von Danwitz et al. 2016) and is a sensitive and thus suitable candidate for testing novel feed formulations. Therefore, the present study aims to evaluate the effects of two novel feed formulations for sustainable turbot production, with moderate fishmeal replacement and using feed ingredients of terrestrial animal and plant origin, on the growth and feed performance, apparent digestibility of nutrients, energy storage and apparent availability of minerals and trace elements.

Material and methods

Experimental diets

All experimental diets were formulated to be isonitrogenous (530 g kg−1). Due to species' behaviour and size, 3 mm pellets with positive buoyancy (floating pellets were manufactured by extrusion at SPAROS LDA (Olhão, Portugal). All diets, including the control diet, were produced using the same facility and extrusion parameters to minimise technological differences. There were three treatments, including two novel formulations and one control diet, which was mimicking a typical current commercial formulation used for turbot. In the control diet, the main protein sources were fishmeal (500 g kg−1), wheat gluten (110 g kg−1) and soy protein concentrate (100 g kg−1). In the two experimental diets, the commercial fishmeal was fully replaced with fish by-products (meal and hydrolysates), and the overall fish-derived content was reduced by 20% to 400 g kg−1. The remaining protein was sourced with emerging ingredients such as insect meal, single cell meal and algae meal. Soy-derived ingredients were replaced by pea protein and pea starch. Furthermore, in all experimental diets, DHA-rich algae and rapeseed oil replaced 60% of fish oil. The content of the respective experimental diets as well as the control diet is shown in Tables 1 and 2. Once the experimental feeds were produced, they were delivered from Portugal to the experimental facility at the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI) in Bremer- haven (Germany). Before and during the trials, the feed was stored at 4 °C to ensure continuous quality of the diets throughout the feeding experiment.

Experimental setup

Juvenile turbot (*Scophthalmus maximus*) were purchased from France Turbot (L'Épine, France), transferred in specific transport containers overland to the recirculating aquaculture systems (RAS) of the Centre for Aquaculture Research (ZAF) at AWI, acclimated to the RAS for 2 weeks prior to starting the 16 week (112 days) experimental trial. A total of 750 turbots with a mean weight (± SD) of 20.2 ± 0.4 g and a mean total length of 10.1 ± 0.1 cm were randomly distributed into 15 tanks (50 fish per tank, 5 tanks per diet). The RAS consisted of 36 tanks, each with a bottom area of 1 m2 and a volume of approx. 700 L. The condition of the process water was monitored constantly with a SC 1000 Multiparameter Universal Controller (Hach Lange GmbH, Germany), and the nutrient concentration was measured with the QuAAtro39 AutoAnalyzer (SEAL Analytical, Germany) twice a week (see Table 3).

The fish were fed twice a day (9 am and 2 pm) ad libitum. After the fishn were fed in the afternoon (30 min later), the remaining pellets were netted (mesh size 1 mm) from the tanks, dried for 24 h at 50 °C and weighed. To account for potential weight loss of the non-eaten pellets, duplicates of each experimental diet (2 g each) were incubated at 16 °C and 100 cycles per minute in 100 mL water which was taken from the experimental recirculation system (30 ‰ salinity) (Obaldo et al. 2002). After 30 min, the content was sieved (mesh size 1 mm), the collected pellets were dried for 24 h at 50 °C and weighted. The weight loss was used to calculate the loss factor for later correction of the recovered non-eaten pellets (see Formula (6)).

Table 1 Formulation (%) of the experimental diets for juvenile turbot (*Scophthalmus maximus*)

Ingredients	Control	PAP	PLANT
Fishmeal[1]	50.00	0.00	0.00
Fishmeal (by-product)[2]	0.00	35.00	35.00
Fish hydrolysate (by-product)[x]	0.00	5.00	5.00
Insect meal (*Hermetia illucens*)[x]	0.00	5.00	5.00
Porcine hemoglobin[3]	0.00	2.50	0.00
Poultry meal[4]	0.00	10.20	0.00
Microbial protein meal (methanotrophic bacteria)[x]	0.00	2.50	2.50
Yeast protein meal (*Saccharomyces cerevisiae*)[x]	0.00	2.50	2.50
Microalgae meal (*Arthrospira platensis*)[1]	0.00	0.00	2.00
Microalgae meal (*Chlorella vulgaris*)[5]	0.00	0.00	0.50
Microalgae meal (*Tetraselmis chuii*)[5]	0.00	0.00	0.20
Soy protein concentrate[6]	10.0	0.00	0.00
Pea protein concentrate[7]	0.00	5.00	12.40
Wheat gluten[7]	11.00	10.00	11.50
Soybean meal[8]	4.00	0.00	0.00
Wheat meal[9]	8.00	0.00	0.00
Pea starch[10]	4.00	8.99	8.89

Fish oil[1]	11.60	4.64	4.64
DHA-Rich algae (*Schizochytrium*)[11]	0.00	1.08	1.08
Rapeseed oil[12]	0.00	3.44	4.64
Rapeseed lecithin[13]	0.00	0.80	0.80
Vitamin and mineral premix[14]	1.00	1.00	1.00
Vitamin C[15]	0.05	0.05	0.05
Vitamin E[15]	0.05	0.05	0.05
Betaine HCl[16]	0.00	0.50	0.50
Macroalgae mix[17]	0.00	0.50	0.50
Antioxidant[18]	0.18	0.18	0.18
Sodium propionate[19]	0.10	0.10	0.10
L-Tryptophan[20]	0.00	0.15	0.15
DL-Methionine[21]	0.00	0.30	0.30
L-Taurine[16]	0.00	0.50	0.50
Yttrium oxide[22]	0.02	0.02	0.02

Control commercial-like formulation, *PAP* processed animal protein, *PLANT* plant-based protein

[x] not disclosed; [1] Sopropêche, France; [2] Conserveros Reunidos S.A., Spain; [3] SONAC BV, The Nether- lands; [4] SAVINOR UTS, Portugal; [5] Allmicroalgae, Portugal; [6] ADM, The Netherlands; [7] Roquette Frères, France; [8] CARGILL, Spain; [9] Casa Lanchinha, Portugal; [10] COSUCRA, Belgium; [11] Alltech, Ireland; [12] Henry Lamotte Oils GmbH, Germany; [13] Novastell, France; [14] DL-alpha tocopherol acetate, 255 mg; sodium menadione bisulphate, 10 mg; retinyl acetate, 26,000 IU; DL-cholecalciferol, 2500 IU; thiamine, 2 mg; riboflavin, 9 mg; pyridoxine, 5 mg; cyanocobalamin, 0.5 mg; nicotinic acid, 25 mg; folic acid, 4 mg; L-ascorbic acid monophosphate, 80 mg; inositol, 17.5 mg; biotin, 0.2 mg; calcium pantothenate, 60 mg; choline chloride, 1960 mg. Minerals (g or mg kg^{-1} diet): copper sulphate, 8.25 mg; ferric sulphate, 68 mg; potassium iodide, 0.7 mg; manganese oxide, 35 mg; organic selenium, 0.01 mg; zinc sulphate, 123 mg; calcium carbonate, 1.5 g; excipient wheat middlings; [15] DSM Nutritional Products, Switzerland; [16] ORFFA, The Netherlands; [17] Ocean Harvest, Ireland; [18] Kemin Europe NV, Belgium; [19] Disproquímica, Portugal; [20] Ajinomoto EUROLYSINE S.A.S, France; [21] EVONIK Nutrition & Care GmbH, Germany; [22] Sigma Aldrich, USA

Table 1 Chemical composition of the experimental

Moisture (%)	4.1	7.3	6.7
Crude protein (%)	52.9	52.8	52.8
Crude lipid (%)	16.5	16.2	18.1
Ash (%)	7.1	10.5	9.9
Gross energy (MJ kg^{-1})	23.1	20.8	21.2
Minerals and trace elements Calcium (Ca; g kg^{-1})	8.1	22.1	19.1
Potassium (K; g kg^{-1})	4.0	7.4	7.6
Magnesium (Mg; g kg^{-1})	1.8	1.7	1.9
Sodium (Na; g kg^{-1})	4.7	6.8	7.6
Phosphorus (P; g kg^{-1})	10.1	14.5	14.5
Ca/P ratio	0.8	1.5	1.3
Arsenic (As; mg kg^{-1})	7.1	6.1	5.8
Copper (Cu; mg kg^{-1})	29.4	18.6	19.1
Iron (Fe; mg kg^{-1})	278.9	347.3	319.6
Manganese (Mn; mg kg^{-1})	71.8	90.6	69.8
Zinc (Zn; mg kg^{-1})	206.9	174.5	186.3
Amino acids (%)			
Arginine (Arg)	3.55	3.24	3.49
Histidine (His)	1.24	1.18	1.17
Isoleucine (Ile)	2.07	1.95	2.16
Leucine (Leu)	3.26	3.26	3.27
Lysine (Lys)	3.13	3.33	3.34
Threonine (Thr)	2.09	1.97	2.02

Tryptophan (Trp)	0.23	0.28	0.28
Valine (Val)	2.09	2.35	2.31
Methionine (Met)	1.07	1.14	1.21
Cysteine (Cys)	0.26	2.34	0.28
Phenylalanine (Phe)	2.35	0.26	2.39
Tyrosine (Tyr)	1.99	1.97	2.01
Alanine (Ala)	2.25	2.58	2.43
Glycine (Gly)	2.42	2.52	2.16
Proline (Pro)	3.02	2.66	2.57
Serine (Ser)	2.28	2.11	2.13
Taurine (Tau)	0.84	0.84	0.80

Control commercial-like formulation, PAP processed animal protein, PLANT plant-based protein. Values are expressed as means from duplicates

Measurements and sampling

Fish were weighed to 0.2 g precision and measured in length to 0.5 cm precision every 4 weeks. At the end of the 16-week trial, 6 individuals from each of the 15 tanks were sampled, from which 3 fish were used for tissue sampling to determine the energy reserves and 3 fish per tank were sampled as whole fish for proximate and mineral analysis. Fish were anaesthetized with 500 mg L−1 tricaine methanesulfonate (MS-222; Sigma Aldrich, Germany). After recording weight (precision 0.01 g) and length (precision 0.5 cm), fish were sacrificed and tissues (liver and fillet without skin) were rapidly sampled on ice. The liver of three fish per tank (n = 15 fish per diet) was weighted with 0.0001 g precision to determine the hepato-somatic index (HSI). Both tissues were frozen in liquid nitrogen and stored at− 80 °C until further analysis. For digestibility analysis, the faeces were sampled by stripping anaesthetized fish and pooled from one tank, centrifuged at 4 °C and 3,000 × g for 5 min, and the pellets were frozen at − 80 °C until further analysis. To gain sufficient tissue mass, the whole fish bodies were pooled (from the 3 fish taken per tank), cut into small pieces and stored at − 20 °C until further analysis.

Chemical analysis of diets, whole body and faeces

The chemical analysis of the diets was conducted in duplicates (see Table 2) and of the whole body and faeces as pooled replicates per tank (n = 5 tanks per diet). The whole body samples were minced frozen using a meat grinder (MADO Primus, Germany), refrozen at − 20 °C and then freeze-dried for 48 h. The samples of the experimental diets and faeces were freeze-dried for 24 h. The experimental diets and whole body samples were further homogenised in a knife grinder (5000 rpm, 30 s, Grindomix GM 200, Retsch, Germany).

The moisture content, ash, crude protein, crude lipid and energy of the experimental diets, whole body fish and faeces was determined after AOAC (1980). Moisture content of the feeds was determined by drying the samples at 105 °C for 24 h. The moisture con- tent of the whole body and faeces was determined by freeze-drying. Total ash content was determined by combustion of the samples in a muffle oven at 550 °C for 6 h. The total nitrogen in the feed and whole body samples was determined following the automated Kjeldahl Method. Due to small sample volume in the faeces samples, the total nitrogen was determined after the Dumas method. For all samples, the measured total nitrogen was converted to equivalent crude protein (%) by the numerical factor of 6.25. Crude lipid was determined by acid hydrolysis. Gross energy was measured in an adiabatic bomb calorim- eter (Model 6100; Parr Instrument, Germany).

For the analysis of the mineral content, 0.2 g of freeze-dried and homogenised samples of the experimental diets, whole body and faeces was digested in 3 mL nitric acid (HNO_3) (65%, trace grade) in a microwave oven (CEM MARS5, Germany) according to DIN EN 13,805 (2014). After digestion, the samples were diluted with Milli-Q water to 50 mL. Calcium, potassium, magnesium, phosphorus, arsenic, copper, iron, manganese, yttrium and zinc concentrations were analysed in an ICP-OES (iCAP7400; Fisher Scientific, Germany). As reference fish muscle (ERM – BB422, EU) was used.

Glycogen and crude lipid content of liver and muscle tissue

Following the procedure described by Keppler and Decker (1988), glycogen content was determined photometrically after enzymatic hydrolysis of glycogen to glucose. Briefly, fish filet and liver samples (3 individual fish per tank; 15 fish per diet in total) were grinded under liquid nitrogen, and approx. 200 mg tissue was homogenised in 5 × volume of ice- cold 0.6 M perchloric acid (PCA) (w:v). After one cycle of 20 s at 6000 rpm and 3 °C using Precellys 24 (Bertin Technologies, France), samples were sonicated for 2 min at 0 °C and 360 W (Branson Ultrasonics Sonifi 450; Fisher Scientifi Germany), and homogenates were immediately divided for the analysis of total and free glucose concentrations. Due to small volume, the individual samples of liver and muscle were pooled (n = 5 tanks per diet) for the crude lipid content. Following the method of Folch et al. (1957) and Postel et al. (2000), the lipids in the muscle and liver tissue were extracted with 2:1 dichloromethane-methanol (v/v) and an aqueous solution of 0.88% KCl (w:v). Crude lipid content was deter- mined gravimetrically to the nearest 0.001 g and calculated as the percentage of lipids of tissue wet weight.

Data analysis (calculations and statistics)

The growth parameters were based on body weight (BW) and body length (BL) and calculated as follows.

$$Weight\ gain\ (WG, g) = BW_{final} - BW_{initial} \quad (1)$$

$$Relative\ growth\ rate\ \ RGR, \%d^{-1} = 100 \times \left(e^{\frac{ln\left(BW_{final}\right) - \ln\left(BW_{initial}\right)}{feeding\ days}} - 1\right) \quad (2)$$

$$Condition\ factor\ (CF) = 100 \times \frac{BW}{BL3} \quad (3)$$

$$Hepato - somatic\ index\ (HSI) = 100 \times \frac{liver\ weight}{BW\ final} \quad (4)$$

The feed performance parameters, daily feed intake (DFI) and feed conversion ratio (FCR) were based on the feed intake (FI) in g of the off ed amount of feed and the uneaten feed, which is corrected by the soluble loss factor. Total FI and WG for FCR were corrected for the lost biomass through mortalities and sampling.

Annex

$$Total\ Feed\ Intake\ (FI_{total}, g) = Feed_{offered} - (Feed_{uneaten} \times factor_{soluble\ loss}) \quad (5)$$

$$factor_{soluble\ loss} = 1 + [1 - (\frac{Feed\ final}{Feed\ initial})] \quad (6)$$

$$Daily\ feed\ intake\ (DFI,\ \%\ BW\ d^{-1}) = 100 \times FI_{total} \quad \frac{BW_{final} + BW_{initial}}{2} \quad (7)$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{FI\ total}{WG} \quad (8)$$

$$Protein\ efficiency\ ratio\ (PER) = \frac{weight\ gain}{crude\ protein\ intake} \quad (9)$$

The apparent digestibility (ADC) of the dietary nutrients and the apparent availability (AA) of minerals were based on the amount of the inert yttrium marker in the diet and faeces and the respective nutrient or element in faeces and diets.

$$ADC\ dry\ matter\ (\%) = 100 - (100 \times \frac{yttrium_{diet}}{yttrium_{faeces}}) \quad (10)$$

$$ADC\ nutrient\ (\%) = 100 - (100 \times \frac{yttrium_{diet}}{yttrium_{faeces}} \quad (11)$$

$$AA\ (\%) = 100 - (100 \times \frac{yttrium_{diet}}{yttrium_{faeces}} \quad (12)$$

The glycogen content was calculated based on the concentration of total glucose (*ctotal glucose*) subtracted by the concentration of free glucose (*cfree glucose*).

$$= (\Delta A \times V \quad \frac{\times\ d \times V_{sample}}{}\ \times DF \quad (13)$$

whereas A = the change in absorption, Vassay total = the total measurement volume of the assay (ml), = the coefficient of extinction at 339 nm (6.3 mL µmo−1 cm−1), d = the thickness of layer for the cuvette (1 cm), Vsample = the sample volume (mL), DF = the dilution factor and ctissue = the concentration of tissue wet weight in crude extract (mg mL−1).
The glucose concentration was converted to glycogen content using the molecular weight of the glucosyl moiety in glycogen with Mr = 162 g mol−1.

$$Glycogen\ content\ (mg\ g^{-1} wet\ weight\ tissue) = (c \quad (14)$$

Statistical analysis

Statistical analysis was conducted with Sigma Plot (12.5, Systat Software, Germany). One- way analysis of variance (ANOVA) was used to determine significant differences between the treatments. Whenever there were statistically significant differences, an all pairwise multiple comparison procedure was performed using the Holm-Sidak method (overall significance level p = 0.050) to find the difference within the treatments. Values are given as means ± standard deviations.

Results

Growth and feed performance

The experimental feed formulations did not significantly affect the growth of the juvenile turbots, and the survival during the experimental period was high with 0% mortalities (Table 4). After 16 weeks, the fish from the control group increased their weight 3.25 fold (65 g), whereas the fish fed with the experimental diets only increased their weight 3.10 fold (by an average of 62 g). The fish accepted all diets with a daily feed intake (DFI) of 0.99 ± 0.03% BW d^{-1} (n = 15 tanks). The feed conversion ratio (FCR) in the fish from the control group was significantly lower than in the fish from the PAP group, with no significant differences to the fish from the PLANT group. The protein efficiency ratio (PER) was significantly higher in the fish from the control group than in the PAP group with no significant differences to the fish from the PLANT group. The condition factor (CF) was significantly affected by the experimental diets (Table 4). The CFs of fish from PLANT group were significantly lower than of the fish in the control and the PAP group. However, the CF significantly increased in all treatments from the initial to the final (t-test; p = < 0.001).

Table 4 Performance parameters of the juvenile turbot (*Scophthalmus maximus*) fed the experimental diets for 16 weeks ($n = 5$ tanks per diet)

	Control	PAP	PLANT	*F*	*p*
Initial body weight (g)	20.2±0.3	20.1±0.5	20.4±0.4	0.402	0.677
Final body weight (g)	85.2±9.7	82.9±6.1	82.1±9.5	0.183	0.835
Weight gain (g)	65.0±9.5	62.8±5.9	61.7±9.2	0.203	0.819
Relative growth rate (% d^{-1})	1.29±0.09	1.27±0.06	1.25±0.09	0.293	0.751
Daily feed intake (% BW d^{-1})	0.98±0.03	1.01±0.04	1.00±0.03	0.985	0.402
Feed conversion ratio	$0.87±0.03^{b}$	$0.92±0.03^{a}$	$0.90±0.02^{ab}$	5.059	0.026
Protein efficiency ratio	$2.17±0.07^{a}$	$2.06±0.06^{b}$	$2.10±0.05^{ab}$	4.031	0.046
Initial condition factor	1.96±0.02	1.94±0.02	1.95±0.03	0.767	0.486
Final condition factor	$2.11±0.01^{a}$	$2.10±0.01^{a}$	$2.07±0.02^{b}$	7.750	0.007

Control commercial-like formulation, *PAP* processed animal protein, *PLANT* plant-based protein. Values are expressed as means±SD, and values with different letters within the same line are significantly different ($p<0.050$), *F* and *p* values from one-way ANOVA.

Table 5 Proximate whole body composition on wet weight basis and apparent digestibility coefficient of juvenile turbot (*Scophthalmus maximus*) fed the experimental diets for 16 weeks ($n = 5$ tanks per diet)

	Control	PAP	PLANT	*F*	*P*
Moisture (%)	74.1 ± 1.3	76.6 ± 0.9	74.4 ± 3.5	1.802	0.207
Crude protein (%)	16.2 ± 1.1	15.2 ± 0.8	16.1 ± 2.6	0.491	0.624
Crude lipid (%)	4.5 ± 0.4^{a}	3.5 ± 0.4^{b}	3.9 ± 0.5^{ab}	6.585	0.012
Ash (%)	3.7 ± 0.3	3.9 ± 0.1	4.3 ± 0.6	3.039	0.086
Gross Energy (MJ kg^{-1})	5.2 ± 0.6	4.6 ± 0.2	5.3 ± 0.8	2.130	0.162
Apparent digestibility coefficient					
Dry matter (%)	83.2 ± 1.1^{a}	77.1 ± 1.7^{b}	77.2 ± 1.9^{b}	23.103	<0.001
Crude protein (%)	92.0 ± 0.5^{a}	89.8 ± 0.7^{b}	89.7 ± 0.7^{b}	22.626	<0.001
Gross Energy (%)	85.2 ± 0.8	86.4 ± 2.2	86.3 ± 1.1	1.027	0.388

Control commercial-like formulation, *PAP* processed animal protein, *PLANT* plant-based protein. Values are expressed as means ± SD, and values with different letters within the same line are significantly different ($p < 0.050$), *F* and *p* values from one-way ANOVA

Whole body composition, apparent digestibility and energy reserves

The whole body composition with moisture content, crude protein, ash content and energy content did not differ significantly between fish fed the different diets (Table 5). Fish from the PAP group had a significantly lower crude lipid content than the fish from the control group with no significant differences to the fish from the PLANT group. The apparent digestibility coefficients (ADC) of dry matter and crude protein were significantly higher in the fish from the control group compared to the fish from the experimental groups, whereas the ADC of energy was not affected (see Table 5).

The hepato-somatic index (HSI) of fish fed the control diet was significantly higher than that of the fish from the experimental groups (Table 6). The hepatic glycogen was significantly higher in the fish fed the control than in the fish fed the PLANT diet, with no significant difference to the fish from the PAP group. The hepatic lipid content, glycogen, and lipid content in the muscle of turbot showed no significant differences between groups (see Table 6).

Mineral analysis of the diets, mineral balance and apparent availability

The mineral content of the whole body showed no significant differences in the fish from all diets, except for arsenic and copper concentration in the PLANT-feeding fish that was significantly higher than in the control fish, with no significant differences to PAP (Table 7).

For all analysed minerals, the apparent availability (AA) was highest in the control diet compared to the experimental diets, except for potassium and sodium, where the AA in the control was lowest (Table 7). No significant differences were found in the availability of calcium, arsenic and zinc. The potassium and sodium availability in the control was significantly lower than in the experimental diets. In contrast, the availability of magnesium, copper and iron was in the control significantly higher than in the experimental diets. The phosphorus availability was in the control diets significantly higher than in the PLANT diet, with no significant differences to the PAP diet. The manganese availability was in the PLANT diet significantly lower than in the control and PAP diet.

Table 6 Hepato-somatic index ($n=15$ fish per diet), glycogen in wet tissue ($n=15$ fish per diet) and lipid content in dry tissue ($n=5$ tanks per diet) in the liver and muscle of juvenile turbot (*Scophthalmus maxi- mus*) fed the experimental diets for 16 weeks

	Control	PAP	PLANT	*F*	*P*
Hepato-somatic index	1.8 ± 0.3^{a}	1.5 ± 0.3^{b}	1.5 ± 0.3^{b}	4.177	0.022
Liver glycogen (mg g^{-1})	63.7 ± 23.9^{a}	48.0 ± 17.2^{ab}	46.4 ± 16.0^{b}	3.666	0.034
Liver lipid (mg g^{-1})	452.0±56.5	486.6±54.9	527.2±64.8	2.044	0.172
Muscle glycogen (mg g^{-1})	1.7±0.7	1.8±0.7	2.1±0.7	1.411	0.255
Muscle lipid (mg g^{-1})	64.3±20.6	90.8±37.4	77.0±35.2	0.861	0.447

Control commercial-like formulation, *PAP* processed animal protein, *PLANT* plant-based protein. Values are expressed as means ± SD; values with different letters within the same line are significantly different ($p<0.050$), *F* and *p* values from one-way-ANOVA

Table 7 Analysed concentrations on wet weight basis in the whole body and apparent availability (AA) of minerals and trace elements in turbot (*Scophthalmus maximus*) fed the experimental diets for 16 weeks ($n=5$ tanks per diet)

Calcium (Ca; g kg^{-1})	10.41±2.16	10.02±1.91	12.40±2.25	1.832	0.202
Potassium (K; g kg^{-1})	2.79±0.35	3.02±0.22	3.13±0.48	1.061	0.376
Magnesium (Mg; g kg^{-1})	0.33±0.05	0.35±0.02	0.39±0.05	3.138	0.080
Sodium (Na; g kg^{-1})	1.52±0.21	1.63±0.09	1.77±0.20	2.305	0.142
Phosphorus (P; g kg^{-1})	6.34±1.22	6.48±0.89	7.70±1.21	2.254	0.147
Ca/P ratio	1.64±0.04	1.54±0.08	1.61±0.07	2.735	0.105
Arsenic (As; g kg^{-1})	0.72 ± 0.09^{b}	0.83 ± 0.10^{ab}	0.91 ± 0.08^{a}	5.642	0.019
Copper (Cu; mg kg^{-1})	0.48 ± 0.06^{b}	0.53 ± 0.02^{ab}	0.60 ± 0.08^{a}	4.288	0.021
Iron (Fe; mg kg^{-1})	4.74±0.7	7.37±2.52	6.67±1.12	3.430	0.066
Manganese (Mn; mg kg^{-1})	13.25±3.70	11.25±1.59	15.27±2.64	2.611	0.114
Zinc (Zn; mg kg^{-1})	11.22±1.22	11.35±0.61	13.23±1.85	3.608	0.059
Apparent availability					
Calcium (Ca; %)	58.7±14.9	45.6±16.5	37.7±25.8	1.451	0.273
Potassium (K; %)	88.1 ± 1.2^{b}	93.4 ± 1.1^{a}	94.0 ± 0.9^{a}	46.210	<0.001
Magnesium (Mg; %)	-61.7 ± 22.6^{a}	-151.5 ± 37.6^{b}	-142.1 ± 37.4^{b}	10.992	0.002
Sodium (Na; %)	-30.6 ± 37.5^{b}	13.1 ± 19.2^{a}	30.9 ± 5.5^{a}	8.323	0.005
Phosphorus (P; %)	88.1 ± 1.9^{a}	78.7 ± 7.2^{ab}	77.8 ± 7.1^{b}	4.578	0.033
Arsenic (As; %)	91.8±2.0	90.4±2.9	90.1±2.1	0.751	0.493
Copper (Cu; %)	62.4 ± 5.4^{a}	36.0 ± 2.8^{b}	41.9 ± 3.5^{b}	58.949	<0.001
Iron (Fe; %)	46.9 ± 4.3^{a}	17.4 ± 3.6^{b}	17.7 ± 7.1^{b}	52.347	<0.001
Manganese (Mn; %)	72.5 ± 11.5^{a}	52.8 ± 7.3^{a}	32.4 ± 23.5^{b}	8.156	0.006
Zinc (Zn; %)	46.0±6.6	27.3±15.9	26.2±21.3	2.484	0.125

Control commercial-like formulation, *PAP* processed animal protein, *PLANT* plant-based protein. Values are expressed as means ± SD, and values with different letters within the same line are significantly different ($p<0.05$), *F* and *p* values from one-way ANOVA

Discussion

In the present study, the more sustainable feed formulations, in which fishderived ingredients were reduced by 20%, resulted in juvenile turbot with similar growth comparing to the control (commercial-type feed) group. This is congruent with literature where decreased growth and feed performance were observed when more than 30–35% of fishmeal was replaced by processed animal protein (Dong et al. 2016), insect meal (Kroeckel et al. 2012) and plant protein (Bian et al. 2017; Bonaldo et al. 2015; Burel et al. 2000a; Fournier et al. 2004; Hermann et al. 2016; von Danwitz et al. 2016). Nevertheless and not unexpected, compared to controls, fi that were fed the slightly leaner experimental diets were less capable of building up energy reserves as the hepato- somatic index (HSI) and the slightly lower liver glycogen show, possibly augmented by a slightly lower apparent digestibility of dietary protein (2%) and energy (3%) of the control diet compared to the experimental diets. The fish in the PAP group had the poorest feed conversion, whereas other parameters showed no clear picture. In any case, the results of present study indicate that successful substitution of traditional fishmeal and fish oil can be achieved in turbot. It can further be speculated that an equal fraction of digestible energy contents between the different diets would have led to an even better similarity in fish performance between the diets of the GAIN project alternative formulations as it was shown with similar formulation in Gilthead seabream (Aragao et al. 2020). Even though the crude lipid content in the PLANT diet was 2% higher than in the control and PAP diet, a possible effect of this on the turbot can be negligible. The crude protein level (52%) used in present study is sufficiently high for turbot to minimise possible effect of the differing crude lipid level as previously observed in juvenile turbot (Sevgili et al. 2014).

Growth and feed performance

The relative growth rate (RGR) was similar between all groups, on average 1.27 ± 0.08% d−1 (n = 15 tanks) indicating that the different diets did not affect turbots' energy allocation with respect to growth performance. In the present study, the RGR was higher by 0.01 percentage points compared to the specific growth rate (SGR), which is widely used in literature (i.e. Arnason et al. 2009; Bonaldo et al. 2011; Burel et al. 1996; Nagel et al. 2012). However, it is incorrect in concept to express the SGR as a percentage increase in daily weight, and therefore, the RGR is used instead (Hardy and Barrows 2003). The presented RGR results were lower than those of similar sized turbot based on the majority of available literature data (Arnason et al. 2009; Burel et al. 1996; Fuchs et al. 2015; Imsland et al. 1996; Nagel et al. 2017) but moderate to high compared to turbot in commercial RAS (Baer et al. 2011).

The feed conversion ratio (FCR) and protein efficiency ratio (PER) were significantly better in the control than in the PAP group. However, the differences between the FCRs in all diets were small with a mean value of 0.90 ± 0.03 (n = 15), and the daily feed intake (DFI) was approximately 0.99% BW d−1 resulting in a RGR in the expected range (Burel et al. 1996). Furthermore, the turbot strain used in the present study might have a lower growth rate per se, as turbot exhibit counter gradient variation (Imsland et al. 2000). Strains from lower latitudes, such as France, show generally lower growth and feed efficiency compared to populations from higher latitudes, such as Norway and Iceland (Imsland et al. 2001).

In present study, the differences in FCR might be overestimated due to the higher moisture content in the experimental diets compared to the control diet. The same pattern as for the FCR was observed in the protein efficiency ratio, with the highest value for the control, followed by PLANT and PAP. In line with literature, in the present study, the apparent digestibility coefficient (ADC) of protein in turbot decreased (> 90% in the control group with 450 g kg−1 fishmeal) when the fishmeal inclusion level is reduced (Bai et al. 2019; Bonaldo et al. 2011; Li et al. 2019; Liu et al. 2014b; Regost et al. 1999). When combining all feed performance indicators, the fish from the control group had the best performance followed by the PLANT group and the PAP group where fish showed the lowest performance.

Even though the condition factor (CF) of turbot from the PLANT group showed statistically a difference to the control and PAP group (2.07 vs. 2.11 and 2.10, respectively), the physiological relevance is minor and does not indicate poorer nutritional status. CFs above 2 indicate an overall good nutritional status of the fish, as presented CFs are similar to values of in previous studies (Fuchs et al. 2015; Nagel et al. 2017; von Danwitz et al. 2016; Wanka et al. 2019; Weiß and Buck 2017). In previous studies, a reduced CF was observed in turbot fed with plant-based diets (Bonaldo et al. 2015) and insect meal-based diets (Kroeckel et al. 2012) at a substitution/replacement level of more than 55%. In line with present study, reduced CF in fish fed with different PAPs was not observed in previous studies for European sea bass (Campos et al. 2017), Gilthead seabream (Karapanagiotidis et al. 2019) and rainbow trout (Lu et al. 2015). However, this might be biased by a lack of studies on this feed ingredient.

Nutritional and energy status

In this study, fish from the control group had a significantly higher HSI than the fish from the two experimental groups (1.8 vs. 1.5 and 1.5, respectively). The HSI of the control group is good for juvenile turbot (Bonaldo et al. 2015; Dietz et al. 2012; Nagel et al. 2017). The hepatic glycogen of the control, PAP and PLANT fed turbot (63.7 mg g−1, 48.0 mg g−1 vs. 46.4 mg g−1, respectively) followed a similar pattern indicating a positive correlation between glycogen as energy reserve in a good nutritional status and the HSI (Guerreiro et al. 2015a; Liu et al. 2014a; Miao et al. 2016; Zeng et al. 2015). Hepatic glycogen serves in many fish species as an energy reserve, and high glycogen deposition leads to increased liver weight in many fish species (Hemre et al. 2002). The liver lipid content was not affected by the diet, which is in line to a study by Guerreiro et al. (2015b) on European seabass that were fed with plant protein compared to fish protein. The effects of the diet on the hepatic lipid content might be minor since turbot does not store excess dietary lipid in the liver or muscle (Leknes et al. 2012; Liu et al. 2014a; Regost et al. 2001). The muscle glycogen and lipid content were not affected by the diets, whereas the muscle glycogen (1.9 mg g−1, calculated for all animals, irrespective of diet group) was on the lower range of 1–12 mg g−1 compared to previous studies (Miao et al. 2016; Pichavant et al. 2002; Soengas et al. 1995).

Considering the lower HSI and hepatic glycogen of the PAP and PLANT fed turbot, we can conclude that the experimental diets used in present study did alter the nutritional status of turbot to a certain degree without negatively affecting the growth. The decreased apparent digestibility of the experimental diets might have caused a reduced surplus on energy resulting in slightly smaller liver masses and thus HSI in PAP and PLANT fed tur- bot. Interestingly, it has been shown that the reduction and replacement of fishmeal with alternative feed ingredients could lead to contradicting results. Decreasing fishmeal content may lead to a decreased HSI (Bai et al. 2019; Gu et al. 2017; Kroeckel et al. 2012; von Danwitz et al. 2016; Wanka et al. 2019), unchanged HSI (Bonaldo et al. 2015; Fuchs et al. 2015; Wang et al. 2016; Weiß and Buck 2017) or even increased HSI (Dietz et al. 2012; Fournier et al. 2004; Nagel et al. 2017). This aspect might be worth investigating in more detail to unravel the observed variation in HSI, hepatic glycogen and lipid dependent on alternative feed ingredients.

Mineral balance, utilisation and availability

The concentrations of calcium, potassium, sodium, phosphorus, iron and manganese were lower in the control diet than in the experimental diets. The type of fishmeal used can explain the elevated ash, calcium and phosphorus content in the experimental diets. Fishmeal from fish by-products has a higher ash content containing much calcium and phosphorus due to a higher content of bones compared to traditional fishmeal (Olsen and Hasan 2012). These differences, however, did not significantly affect the concentration of minerals and trace element in the whole body of turbot, which are similar to those of other species (see meta-analysis by Antony Jesu Prabhu et al. 2016). However, the manganese concentration was twice as high as the maximum described for different fish species (Antony Jesu Prabhu et al. 2016) and for turbot in RAS (van Bussel et al. 2014). This might be due to accumulation effects in the whole body, which was already described in turbot (Ma et al. 2015) and Atlantic salmon parr (Lorentzen et al. 1996).

Even though there were no diet-dependent effects on the concentrations of calcium, arsenic and zinc, the apparent availability magnesium, copper and iron were significantly higher in the fish from the control group than in the fish fed with the experimental diets. Furthermore, the apparent availability of phosphorus and manganese was significantly reduced in fish fed the plant-based diet compared to the fish fed the control. Potassium and sodium had a significantly reduced apparent availability in the fish fed the control compared to the fish fed the experimental diets. Substances such as phytate in plant-based feed ingredients are known to bind minerals and, thus, reduce the availability of phosphorus, iron and zinc in fish (Kumar et al. 2012). The inclusion of rape-seed in diets leads to a reduced availability of phosphorus, manganese, iron and zinc but increased copper availability in turbot (von Danwitz et al. 2016). The potassium and sodium availability was in general high and was significantly higher in fish fed the experimental diets than in the control group (93% and 94% in PAP and PLANT vs. 88% in control). In contrast, the potassium availability in rainbow trout was higher in the fishmeal-based diet than in the plant-based diet (Antony Jesu Prabhu et al. 2015, 2018). Since the mineral concentrations in the whole body are similar in the fish from all experimental groups, it can be concluded that the mineral and trace element demand was sufficiently covered and that the elevated mineral concentration in the experimental diets was balanced by elevated excretion rates.

Prediction of feed and production costs

The present results of growth performance indicate that alternative feed formulations can be used in commercial aquaculture for juvenile turbot. Since feed costs are the largest cost factor in the production, small differences in the FCR can balance feed costs and could make more cost efficient formulations attractive. The animal-based formulation (PAP) presented in this study has a lower cost with a commercial margin than the commercial-like control formulation, whereas the plant protein formulation (PLANT) is more expensive (see Table 8). Taking this study's FCRs into consideration, the feed costs to produce one ton of turbot is still lower with the PAP formulation than the control and the PLANT formulation. Feeding juvenile turbot with the PAP formulation could lead to a cost reduction of 10% compared to the control, whereas feeding the PLANT formulation would increase the costs by 12%.

Conclusion

The present study highlighted that fish by-products are a suitable replacement for commercial fishmeal and that protein sources derived from terrestrial plants or animals can replace 20% of the overall fish-derived ingredients without compromising growth performance and body composition of juvenile turbot. These findings are a promising start for further research to find the optimal replacement of marine ingredients, in order to ensure acceptable feed utilisation and deviations from nutritional status. Overall, the alternative diet formulations may produce leaner fish, which have the potential for muscle growth rather than adiposity, and the slightly lowered apparent digestibility of protein suggests that waste production within a commercial aquaculture system would not be much higher than with feeding the control diet. Furthermore, the feed formulation based on processed animal protein (PAP) seems to be an economical feasible alternative for juvenile turbot since the lower feed related production costs balance the slightly poorer feed conversion. Further studies on turbot in the grow-out phase will investigate how a higher fishmeal replacement will affect the performance.

Besides the effects of the alternative feed formulations on fish performance, the economic and environmental benefits of the diets, the consumers' acceptance of the diet formulations need to be considered. Alternative feed ingredients, sourced through circular economy processes, could be more environmentally sustainable (Maiolo et al. 2020) but may also increase production costs. Hereby particularly, insect and algae production could be included in an integrated multi trophic aquaculture

(IMTA) system, which reduces the environmental impact by recycling of nutrients (Barrington et al. 2009; Milhazes-Cunha and Otero 2017). Many consumers are concerned that feed ingredients, such as by-products from terrestrial animals, may not be safe (Glencross et al. 2020). Furthermore, they express the concern that the feed formulations with high levels of plant ingredients might not be species appropriate and impair the animal welfare of cultured fish (Feucht and Zander 2015). Therefore, in addition to the marketing of more sustainable aquaculture products in Europe, such socio-economic aspects need to be considered when developing new and innovative fish diets for commercial important fish species.

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Author contribution CH: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing (original draft), writing (review and editing) and visualisation.
JP: Methodology, validation, investigation and writing (review and editing).
GL: Methodology, validation, formal analysis, resources, writing (review and editing) and supervision.
JJ: Conceptualization, methodology, writing (review and editing), project administration and funding acquisition.
GP: Methodology, resources and writing (review and editing).
LC: Conceptualization, methodology, resources, writing (review and editing), project administration and funding acquisition.
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Data availability Data will be available over the PANGAEA ® Data publisher.

Code availability Not applicable.

Declarations

Ethics approval The experiments were performed under the guidelines of the local authority 'Food surveil- lance, animal welfare and veterinary service (LMTVet)' of the state of Bremen with the permission to carry out animal experiments (500–427-103–1/2019–1-19).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

Annex

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Declaration on the contribution of the candidate to a multi-author article/manuscript which is included as a chapter in the submitted doctoral thesis

Chapter: Publication I (Petereit *et al.* 2022)
Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):
Experimental concept and design: ca. 70%
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Drafting of the manuscript: ca. 100%

Bremen,

Signature:

"I am not what happened to me. I am what I chose to become."

- Carl Gustav Jung

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