Master's thesis

NTNU
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Chemical and nutritional investigation of four little utilized fish species caught on the coast of Mid-Norway

Master's thesis in Food science, sustainability and technology
Supervisor: Jørgen Lerfall
Co-supervisor: Sophie Kendler
May 2022

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Trondheim, May 2022
Frida Walle Thornes

Abstract

The chemical composition of four little utilized fish species captured on the West Coast of Norway was investigated to increase knowledge of the species and determine their nutritional profile. Central fillets of three species of flatfish, flounder (*Platichthys flesus*), lemon sole (*Microstomus kitt*), and megrim (*Lepidorhombus whiffiagonis*), were investigated, as well as one species of skates, thornback ray (*Raja clavata*). The proximate composition of the species was analyzed, as well as the protein and lipid profile, including free and total amino acids and fatty acids. For flatfish species, both upper belly and lower loin fillets were investigated for inhomogeneous distribution of their chemical composition. From the proximate composition, fish had a high protein content, from 17% in flounder and lemon sole to 19% in megrim and 24% in thornback ray. They further had a high water content (from 79% to 82%) and low lipid content (from 0.7% to 1.0%).

From the total amino acid analysis, flounder, megrim, and thornback ray were determined to be protein sources of ”excellent” quality as they obtained digestible indispensable amino acid scores (DIAAS) above 100%. This means their indispensable amino acid distribution covered the nutritional requirements set for adults. Lemon sole contained little methionine (2 mg/g wet weight) and thus obtained a DIAAS of 70%. One dinner serving (200 g) of flounder and megrim covered on average 61% and 68%, respectively, of all the daily indispensable amino acid requirements for an 80 kg adult. The free amino acid analysis showed an abundance of glycine/arginine, alanine, and lysine, with high variance for all species. The species' fatty acid profiles had a high proportion of polyunsaturated fatty acids (from 32% to 49%), where the primary fatty acids were n3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). One dinner size serving (200 g) of flounder, megrim and thornback ray covered on average 106%, 96%, and 91%, respectively, of the daily recommended intake of 500 mg DHA+EPA. The species all displayed excellent nutritional value, and increased consumption of all species can be recommended. Flounder and megrim had the most beneficial nutritional profiles. No difference was found between the flatfish's upper belly and lower loin fillets, so the same nutritional benefits are obtained from consuming all four central fillets of the fish.

Sammendrag

Den kjemiske sammensetningen til fire lite utnyttende fiskearter på vestkysten i Norge ble undersøkt for å øke kunnskapen om artene og undersøke deres næringsinnhold. Tre flatfisker ble undersøkt, skrubbe (*Platichthys flesus*), lomre (*Microstomus kitt*) og glassvar (*Lepidorhombus whiffiagonis*), samt en skate-art, piggskate (*Raja clavata*). Proksimat sammensetning, i tillegg til proteinprofil gjennom totale og frie aminosyrer og fettprofil gjennom fettsyrer, ble analysert. For å undersøke fordelingen av kjemiske komponenter i ulike filleter ble øvre ventrale og nedre dorsale filleter hos flatfiskene også undersøkt. Artene hadde et høyt proteininnhold fra 17% hos skrubbe og lomre til 19% hos glassvar og 24% hos piggskate. De hadde videre et høyt vanninnhold (79% til 82%) og et lavt fettinnhold (0.7% til 1.0%).

Den totale aminosyrer-analysen viste at skrubbe, glassvar og piggskate er proteinkilder av ”utmerket” kvalitet, da de fikk en fordøyelig essensiell aminosyrescore (DIAAS) over 100%. Dette betyr at deres aminosyreninnhold dekker næringsbehovet til voksne for alle essensielle aminosyrer. Lomre inneholdt lite metionin (2 mg/g våtvekt) og fikk derfor en DIAAS på 70%. En middagsporsjon (200 g) av flyndre og glassvar dekket gjennomsnittlig henholdsvis 61% og 68% av behovet for alle daglige essensielle aminosyrer for en 80 kg tung voksen. Den frie aminosyrer-analysen avdekket at glysin/arginin, alanin og lysin var de mest vanlige frie aminosyrene, med stor variasjon mellom individer for alle artene. Artenes fettsyreprofil var alle dominert av flerumettede fettsyrer (32% til 49%), hvor de vanligste flerumettede fettsyrene var n3 fettsyrene dokosaheksaensyre (DHA) og eikosapentaensyre (EPA). En middagsporsjon (200 g) av skrubbe, glassvar og piggskate dekket gjennomsnittlig henholdsvis 106%, 96%, og 91% av daglig anbefalt inntak av 500 mg DHA+EPA. Totalt sett hadde alle artene utmerket næringsinnhold, og økt konsum av alle artene kan anbefales. Skrubbe og glassvar hadde de mest gunstige næringsprofilene. Det ble ikke funnet noen forskjeller mellom øvre ventrale og nedre dorsale filleter hos flatfiskene, og det samme gunstige næringsinntaket oppnås ved konsum av alle de fire filletene.

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Abbreviations

ANOVA	=	Analysis of variance
DHA	=	Docosahexaenoic acid
DIAAS	=	Digestible indispensable amino acid score
DPA	=	Docosapentaenoic acid
EPA	=	Eicosapentaenoic acid
FA	=	Fatty acids
FAA	=	Free amino acids
FAME	=	Fatty acid methyl ester
FAO	=	Food and Agriculture Organization
FID	=	Flame ionization detector
GC	=	Gas chromatography
HPLC	=	High-performance liquid chromatography
IAA	=	Indispensable amino acids
IUCN	=	International Union for Conversation of Nature and Natural Resources
LL	=	Lower loin (fillets)
LUR	=	Little utilized resources
MSC	=	Marine Stewardship Counsil
MSG	=	Monosodium glutamate
MSY	=	Maximum sustainable yield
MUFA	=	Monounsaturated fatty acid
OPTiMAT	=	Optimal utilization of marine food resources
PCB	=	Polychlorinated biphenyls
pp	=	percentage points
PUFA	=	Polyunsaturated fatty acids
SFA	=	Saturated fatty acids
TAA	=	Total amino acids
UB	=	Upper belly (fillets)
ww	=	wet weight

Chapter 1

Introduction

1.1 The need of finding new food resources

By 2050, the demand for food is projected to increase by 50% as the world's population is expected to reach 9.7 billion people [FAO, 2018, UN, 2019]. At the same time, food production accounts for a quarter of total greenhouse gas emissions, and immediate action is needed to reduce climate gas emissions and counter climate change [Vermeulen et al., 2012]. Finding ways to produce more food, while at the same time reducing the climate impact of that food, is a tremendous challenge in the years to come. New food resources must be investigated and researched to enable this.

The ocean, covering 71% of the Earth's surface, is a promising area to find new food resources. While 50% of the world's biomass originates from the ocean, only two percent of human food (in terms of human energy intake) originates from there [Skjermo et al., 2014]. Marine fish generally has a high nutritional value, and increased fish consumption can reduce malnutrition and correct imbalanced diets [FAO, 2020]. Global fish intake has steadily increased over the past 60 years and is projected to further increase in the coming years [FAO, 2020]. Nevertheless, most of the world's fisheries are presently captured at or above maximum capacity and cannot be exploited further [FAO, 2020].

Norway has the second-longest coastline globally and an abundance of marine species. Even so, only around 10% of Norway's 220 marine species have been developed commercially as food [Fiskeridirektoratet, 2022]. To focus on the remaining species, an initiative was started at the end of the 1970s, formally called "LUR" (little utilized resources) [Bjørklund and Henriksen, 2011]. The aim is to improve the utilization of new marine species for food on a commercial scale in Norway. LUR species are characterized as not fully commercially exploited in Norway today, with many species mainly caught as by-catch. LUR species include fish, crustaceans, mollusks, and echinoderms. Despite considerable efforts to develop different LUR species commercially since the start of the initiative, only two species, edible crab (*Cancer pagarus*) and greater Argentine (*Argentina silus*) have been successfully commercialized. Several hurdles to successful

commercialization have been identified, including lacking profitability, non-adapted production and distribution, and lacking markets [Bjørklund and Henriksen, 2011].

The most recent LUR report was published in 2011 and concluded that flatfish were among the species with the most significant potential for successful commercialization [Bjørklund and Henriksen, 2011]. The report presented the following reasons: 1. High probability that stocks are sufficient to allow more capture than what is caught presently. 2. Markets exist, both domestic and abroad, for the distribution of flatfish, and 3. Stakeholders interested in dealing with challenges regarding the value chain exist [Bjørklund and Henriksen, 2011]. Further research and investigations into flatfish were recommended to enable the commercialization of flatfish. Species named in the report included flounder (*Platichthys flesus*), lemon sole (*Microstomus kitt*), and megrim (*Lepidorhombus whiffiagonis*).

1.2 Aims of the thesis

The present thesis is a part of the Optimal utilization of marine food resources - OPTiMAT project at NTNU and has been carried out in close collaboration with one of the project's PhD candidates, Sophie Kendler. The main objective of the present thesis was to increase the knowledge about four little utilized fish species often captured as by-catch by Norwegian fishermen. The increased knowledge could contribute to further focus and investment in these species, enabling successful commercialization. Within the main objective, three sub-objectives were defined:

1. To determine the chemical and nutritional profile of flounder (*P. flesus*), lemon sole (*M. kitt*), megrim (*L. whiffiagonis*), and thornback ray (*Raja clavata*). Moreover, to investigate the nutritional benefits of consuming these species.

2. To study chemical and nutritional differences between the selected species to determine whether some species had a more beneficial nutritional profile than others.

3. To investigate the distribution of nutritional compounds between different parts (belly/loin and upper/bottom fillets) of each species of flatfish.

Chapter 2

Background

2.1 Flatfish (Pleuronectiformes) and skates (Rajiformes)

The investigated species in this thesis belong to the orders of flatfish (Pleuronectiformes) and skates (Rajiformes). Although the two groups are not taxonomically closely related, they take up many of the same ecological niches and resemble each other morphologically with characteristically flat bodies. Both groups are found worldwide in various habitats, with individual species having restricted distributional ranges [Gibson, 2005, p. 51]. The groups are mostly benthic, eating crustaceans, mollusks, and small fish found on the seafloor [Gibson, 2005, p. 190].

2.1.1 Flatfish (Pleuronectiformes)

Flatfish (Pleuronectiformmes) is an order of bony fish with easily recognized asymmetry [Gibson, 2005, p. 35]. Currently, more than 700 species are recognized worldwide, with around 15 species identified off the coast of Norway [Gibson, 2005, p. 51].

Flatfish are the only vertebrates that deviate radically from a bilateral symmetry [Gibson, 2005, p. 10]. This asymmetry is only present in adult fish, as flatfish lay eggs that hatch and develop into symmetrical larvae. The pelagic larvae migrate with the currents into nursery grounds, where they settle and develop into juveniles. During development, the larvae undergo a remarkable metamorphosis where one eye migrates towards the other, and their bilateral sides differentiate [Gibson, 2005, p. 10]. Consequently, flatfish have four separated fillets compared to the two of most other fish.

A metamorphosed flatfish spends most of its time on the sea bottom, where it typically prefers a sandy substrate in which it can bury itself [Gibson, 2005, p. 213]. Adult flatfish consume two types of prey. Some species, like flounder and lemon sole, mostly eat polychaetes and crustaceans, while others, including megrim, mostly eat smaller fish [Gibson, 2005, p. 190]. In temperate waters, flatfish spawn once to twice per year, where they gather in designated spawning areas. The basic anatomy of flatfish is shown in fig. 2.1.

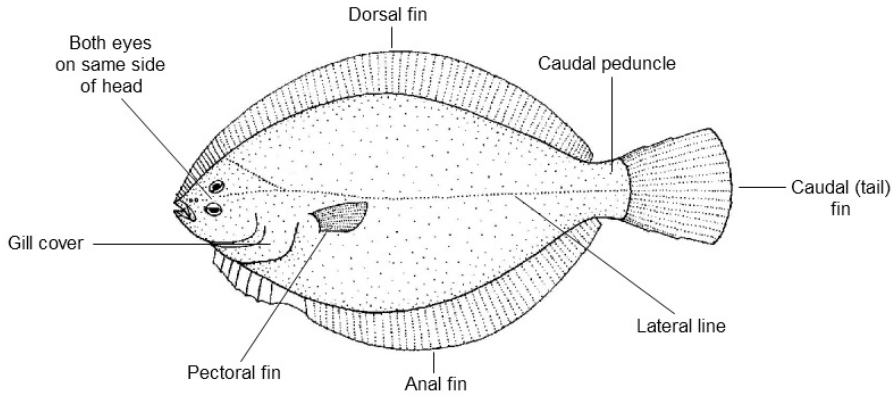

Figure 2.1: The basic anatomy of flatfish. Figure from British Sea Fishing [n. d].

2.1.2 Skates (Rajiformes)

Rays are the largest subgroup of cartilaginous fish, fish that are characterized by their cartilages and exposed gills. Rays comprise 633 valid species and are recognized by a flat disc of a body with an elongated snout and long tail [Heessen et al., 2015, Last and Marshall, 2016, pp. 1, 96]. They vary in length from less than 20 cm to over 6.5 meters and are found worldwide. Rays have a slow growth rate, late maturity, and low fecundity compared to bony fish (Osteichthyes), making ray populations more vulnerable to overexploitation [Santos et al., 2021]. They usually have a varying body form with age and sex and can thus be hard to species characterize and identify [Last and Marshall, 2016, p. 3]. Rays have a high brain-weight to body-weight ratio and are theorized to be among the most intelligent non-mammalian species [Last and Marshall, 2016, p. 6].

One order of rays are skates (Rajiformes), the only egg-laying (oviparous) order [Last and Marshall, 2016, p. 7]. Eleven species of skates have been identified in Norwegian waters [Pethon and Nyström, 2019, p. 164]. Skates are primarily carnivores and feed on crustaceans and fish. They live a dispersed lifestyle for better food availability, but an aggregation of sexes takes place during spawning [Last and Marshall, 2016, p. 8]. The basic anatomy of skates is shown in fig. 2.2.

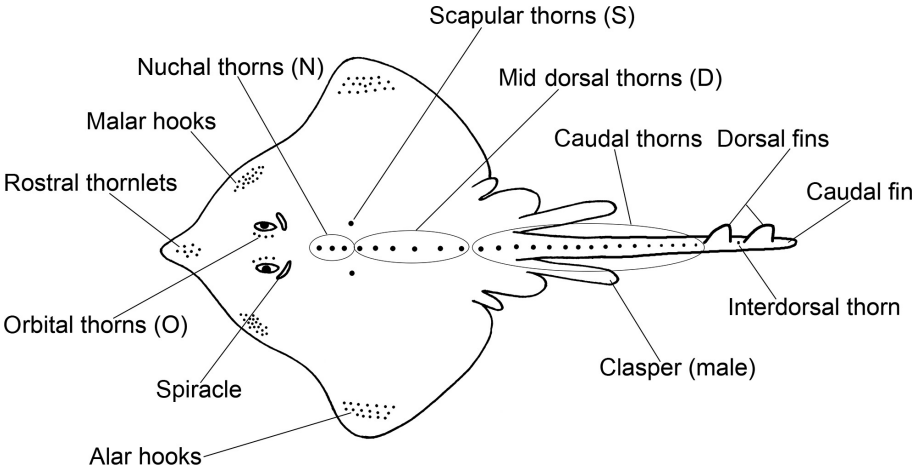

Figure 2.2: The basic anatomy of skates. Figure from Matta et al. (2017).

2.1.3 Species of interest

The species investigated in this master's thesis are European flounder (*P. flesus*), lemon sole (*M. kitt*), megrim (*L. whiffiagonis*), and thornback ray (*R. clavata*). Common for all is that they are of no economic importance to Norwegian fisheries today [Fiskeridirektoratet, 2022]. Illustrations of the species of interest are found in fig. 2.3.

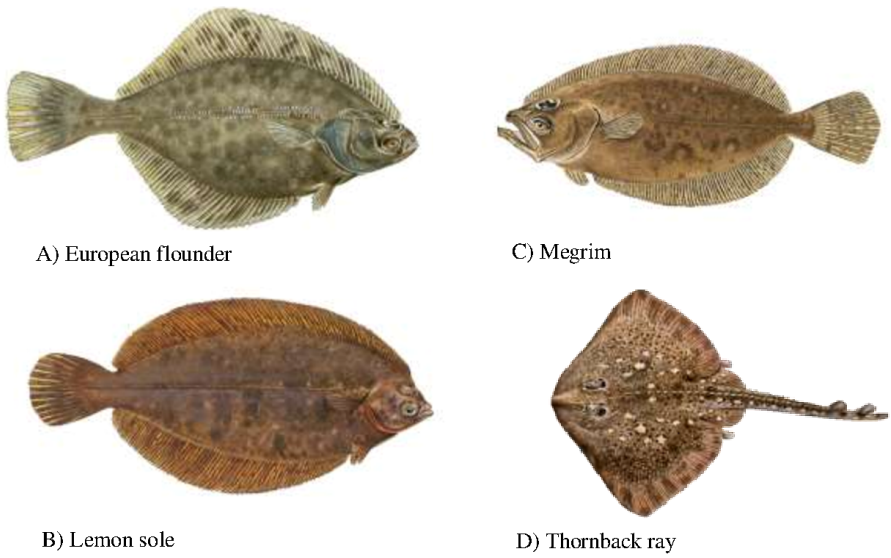

Figure 2.3: Illustrations of the species investigated in the present master thesis. A) European flounder (*Platichthys flesus*), B) lemon sole (*Microstomus kitt*), C) megrim (*Lepidorhombus whiffiagonis*) and D) thornback ray (*Raja clavata*). Figures from Artsdatabanken [n. d].

European flounder (la: *Platichthys flesus*, no: skrubbe) is a lefteye flounder characterized by pointy scales along its lateral line and dorsal and anal fins. Flounders are found all along the coast of Norway, in the Baltic sea, and around the coast of England [Pethon and Nyström, 2019, p. 454]. The species can reach a size of 50 cm and 2.5 kg, with a

typical 5-year old fish around 25 to 35 cm. It reaches maturity around 2 to 3 years of age. The fish can be found from shallow grounds down to 120 meters depths, and they also thrive in brackish waters. Local fisheries of the European flounder are found around the Baltic sea [Pethon and Nyström, 2019, p. 455]. Spawning takes place between February and August.

Lemon sole (la: *Microstomus kitt*, no: lomre) is a left-eye flounder with an oval body and small smooth fish scales. It is commonly found along the Norwegian coast, around the British Islands, and Iceland [Pethon and Nyström, 2019, p. 458]. Lemon sole can reach a length of 66 cm and around one kg weight, attaining maturity around 3 to 6 years of age. As lemon sole matures, it changes habitat and migrates to shallower grounds (10 to 20 m) [Skiftesvik et al., 2003]. This migration makes the capture of adult lemon sole easier, as the capture location can select for larger fish. Lemon sole is caught in large volumes in the North Sea and around Iceland [Pethon and Nyström, 2019, p. 459]. Farming of lemon sole has been discussed in Norway, and a Norwegian pilot study found a promising potential [Mortensen et al., 2004].

Megrim (la: *Lepidorhombus whiffiagonis*, no: glassvar) is a righteye flounder with a prominent underbite and a transparent body where organs can be spotted when held against the light. Megrim is found along the coast of the Atlantic, from Sicily to Iceland, only sparsely populating the Norwegian coast at 50 to 300 m depth. It reaches a typical size of 35 to 45 cm but can grow up to 61 cm [Pethon and Nyström, 2019, p. 449]. Spain is the country that catches the most megrim each year, around 5000 tonnes. Megrim lives on mixed bottoms, often firmly attached to rocks, making them harder to catch by seines [Pethon and Nyström, 2019, p. 449].

Thornback ray (la: *Raja clavata*, no: piggskate) is among the most common skates and is characterized by thorny spines along its entire upper body (hence its name) [Pethon and Nyström, 2019, p. 165]. It is found along the entire Norwegian coast and frequently landed in the Northeast Atlantic, mainly as a by-catch [Heessen et al., 2015, p. 113]. Thornback rays are relatively large skates with a size span of 8 to 115 cm lengthwise, whereas juvenile skates around 40 cm are most common. Females grow bigger than males and can weigh up to 18 kg. The species is found in shallow waters (10 m) to depths of more than 600 m, typically migrating from shallow waters in the summer to deeper waters in the winter [Heessen et al., 2015, p. 115]. Thornback ray reproduces from around March till September by laying eggs that hatch after five months of incubation.

2.1.4 Flatfish and skate capture in Norway

The catch volumes for lemon sole, megrim, flounder, and thornback ray in Norway are low. In 2021, 99 tonnes of lemon sole, megrim, and flounder were caught, making up 1.1% of total Norwegian flatfish catch (excluding Greenland halibut) [Fiskeridirektoratet, 2022]. The catch volumes have remained stable for the species over the past 20 years, with a slight decrease in lemon sole catches and a slight increase in megrim catches, as

seen in fig. 2.4a). The dominant catch gear for flatfish is Danish seine, and in a typical capture, a mix of different benthic flatfish and ray species are caught within the same seine [Fiskeridirektoratet, 2022, Ingolfsson et al., 2016]. The highest amount of Norwegian-landed flatfish is caught in the Norwegian sea and the northern North sea [Fiskeridirektoratet, 2022].

For thornback rays, a total of 11 tonnes were caught in 2021, counting less than one percent of all rays captured in Norway that year. The skates are mainly caught as by-catch [Last and Marshall, 2016, p. 204]. However, as 55% of all skates caught in 2021 were unidentified and misidentifying skates is common, the real catch volumes were possibly higher [Santos et al., 2021]. The capture of thornback rays remained relatively stable between 2000 and 2021, except for the record year of 2020 where 172 tonnes were caught. Most rays are caught in the Barents sea [Fiskeridirektoratet, 2022].

Neither species investigated are regulated by catch quotas. However, minimum sizes are set for megrim (25 cm), lemon sole (25 cm), and flounder (20 cm) [Lovdata, 2015].

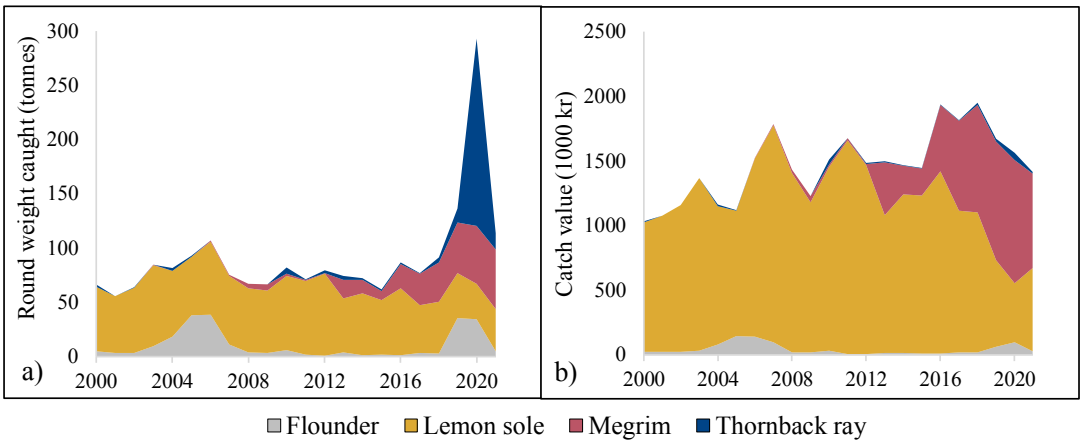

Figure 2.4: Yearly round weight catch (in tonnes) (a) and catch value (in KNOK) (b) of flounder (*Platichthys flesus*), lemon sole (*Microstomus kitt*), megrim (*Lepidorhombus whiffiagonis*), and thornback ray (*Raja clavata*) in Norway between 2000 and 2021. Data from Fiskeridirektoratet [2022].

In terms of primary catch value in Norway, the investigated species were sold for a total of 1.4 MNOK in 2021 fig. 2.4b). This modest income reflects the low catch volumes. Both megrim and lemon sole are relatively highly valued fish, earning an average price per kg of 16.5 and 13.8 NOK/kg in 2021, respectively. This is higher than the average earning of mackerel (10.3 NOK/kg) and saithe (9.4 NOK/kg) for the same year [Fiskeridirektoratet, 2022]. However, flounder and thornback rays suffer from lower average prices per kg, with 5.2 NOK/kg and 0.7 NOK/kg in 2021 respectively. Multiple factors affect the sales price, with weight and size being significant factors. In Britain, lemon sole above 600 g obtains a minimum of 2.5 NOK more per kg than fish below this weight limit [Bjørklund and Henriksen, 2011].

2.1.5 Stock assessment of flatfish and skates

Little research is conducted on stock assessments of flatfish in Norwegian waters, as most are not being commercially fished. Even in areas where the species make up a commercially important catch, stock data is only partially available, and catch recommendations are made through data-limited assessment methods [Lart, 2022]. The latest LUR report from 2011 still pointed out that it is "very likely that the resource baseline is present for a higher exploitation of flatfish (in Norway) than there is today" [Bjørklund and Henriksen, 2011].

The closest assessments of lemon sole, flounder, and thornback ray stocks are conducted in the North Sea [ICES, 2021]. For lemon sole, the fishing pressure in the North Sea has been below maximum sustainable yield (MSY) for the past three years, even though more than 3000 tonnes have been caught yearly, far above catch rates in Norway. For thornback ray, the stock size indicator has steadily increased over the past 20 years, possibly indicating a higher spawning stock biomass today. For flounder, on the other hand, the relative stock size indicator has decreased in the past 20 years, even though fishing pressure has been kept below MSY [ICES, 2021]. For megrim, data-limited stock assessments are not available for the North Sea. The closest stock assessment available is for the sea outside Ireland, where a complete stock assessment concludes with a stock size above trigger levels and will full reproductive capacity in 2021 [ICES, 2021]. It is important to note that the data-limited stock assessments from the North Sea and the coast of Ireland cannot readily be applied to populations in Norway.

Some indicators also highlight the vulnerability of the investigated species. Thornback ray is now listed as near threatened in Europe by the International Union for Conservation of Nature and Natural Resources (IUCN), from an assessment dating back to 2014 [Ellis et al., 2016]. The long lifespans and relatively low productivity of thornback rays make them more vulnerable to overexploitation, with examples of over-exploited rays such as stingrays and wedgefishes in the Indo-Pacific Ocean [Last and Marshall, 2016, Santos et al., 2021, p. 17]. For lemon sole, megrim, and flounder, the species are all characterized as "least concern" by IUCN, from assessments done in 2021, 2020, and 2010 [Abad et al., 2016, Munroe, 2010, 2021].

2.1.6 Flatfish and skates as food

Flatfish have been used as food for millennia and are recognized as having a delicate white flesh and soft texture. Many flatfish species are valued fish and sold for high market prices [Gibson, 2005, p. 347]. Large species of flatfish, including halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*), are being farmed globally to meet consumer demand [Gibson, 2005, 354]. Flatfish fillets are typically prepared and eaten as other white fish and can be used for grilling, pan-frying, baking, and in stews and soups.

The investigated flatfish species are commonly eaten in many parts of the world. Lemon sole is a particularly popular fish in Britain, where it is commonly used in the traditional "fish and chips" dish [Bjørklund and Henriksen, 2011]. Megrim is especially popular in

Spain, where it is highly prized among both restaurants and domestic consumers [Pastoriza et al., 2008]. Although less popular than megrim and lemon sole, flounder is used dried and smoked as a traditional dish in Estonia [Biodiversity, n.d].

The highest consumption of skates takes place in Asia. Skate fillets have a firm texture and mild flavor, and skate wings are a popular food in Asian seafood markets [Last and Marshall, 2016]. In Europe, skates are landed and consumed in the highest amounts in Portugal and France [Santos et al., 2021]. The meat is pinker than among flatfish and can develop a strong ammonia smell [Colakoglu et al., 2011]. The smell is caused by urea in blood and tissues that are converted to ammonia post-mortem and has made skate meat less popular for consumption in Europe. Correct handling of the fish after capture and processing methods such as marinating and smoking can reduce odor development [Colakoglu et al., 2011].

Other parts than the muscle meat can also be used from flatfish and skates. Experiments with gelatin preparation from the skin of flatfish have been successfully carried out, where skins of flatfish (megrim (*L. boscii*) and Dover sole (*Solea vulgaris*) had high gelling ability and also formed thermostable gels [Gómez-Guillén et al., 2002].

2.2 Bottlenecks for better utilization of flatfish and skates

Since the beginning of the LUR project in the 1970s, there have been many attempts to commercialize little utilized marine species [Bjørklund and Henriksen, 2011]. Most of them have failed, which can be linked to non-established value chains and several bottlenecks in the process. The entire marine fish value chain is already established for flatfish and skates. Therefore, resolving and focusing on the bottlenecks will be crucial to commercializing the species in Norway successfully.

Bottlenecks in the commercialization of flatfish are linked to resource availability, capture, regulations, production and technology, and market and distribution. The bottlenecks mentioned are mostly linked to flatfish, as this group has received the most attention. However, the same bottlenecks can also be assigned to skates and several other LUR species.

2.2.1 Resource availability and food safety

Several factors related to resource availability and food safety remain to be determined for the investigated species. Stock assessments for all the investigated species in the Norwegian and Barents sea are lacking, meaning no upper limit on MSY can be determined [ICES, 2021]. An increase in flatfish and skate capture without stock assessments risk overexploitation, and ultimately populations in decline. More information on stocks and populations will also contribute to ensuring profitability. Low and discontinued catch volumes are bottlenecks in flatfish commercialization today and finding capture locations where flatfish aggregate can ensure high catch rates and efficiency [Bjørklund and Henriksen, 2011].

Aspects related to food safety have not been investigated for many flatfish species, including nutritional aspects and heavy metal contamination. Like all food, fish to be eaten and sold in Norway must adhere to Norwegian food law. Fish meat has set limits to environmental toxins and heavy metals, which is 0.30 mg lead/kg wet weight (ww), 0.05 mg cadmium/kg ww, and 1.0 mg mercury/kg ww for *Raja* and *Lepidorhombus* species (thornback ray and megrim) [Lovdata, 2015]. For polychlorinated biphenyls (PCBs) and dioxins, the limits are 75 ng/g ww [Lovdata, 2015]. Flatfish and skates are benthic species at risk of accumulating environmental contaminants present on the seafloor. It is the food producer who is responsible for ensuring the food safety of its product. As such, challenges related to food safety and sustainable harvesting of flatfish should be determined early in the development of the industry.

2.2.2 Capture and regulations

The management of the Norwegian fishing fleet and the regulations of capture methods represent essential bottlenecks in the higher exploitation of flatfish and skates in Norway.

At the beginning of the 2000s, the Norwegian fishing fleet underwent a structurization process that introduced quotas for economically important fish species. The quotas restricted participation in fish capture and led to increased profitability in the industry [Gullestad, 2021]. At the same time, they negatively affected the economic incentive to participate in the capture of less utilized species, including flatfish and skates [Bjørklund and Henriksen, 2011]. As a result, alternative management policies may be necessary to attract fishermen to flatfish capture. An alternative for benthic and little mobile species such as flatfish can be to provide exclusive rights to single actors for restricted geographical areas [Bjørklund and Henriksen, 2011]. This will make profitability more secure for the actors involved, although the alternative management strategies will be resource-demanding to put in place.

Several restrictions exist for flatfish capture methods. Environmental regulations and policies set restrictions for bottom-trawling in Norway, an efficient capture technique that is common in Europe and the United States [Lomeli and Wakefield, 2016, Lovdata, 2005]. The method has a broad negative impact on the seafloor and is therefore not used in flatfish capture in Norway today.

Additionally, in 2004, the Ministry of Trade, Industry, and Fisheries issued the coastal cod protection in Norway after decreasing coastal cod populations since the 1990s [Regjeringen Stoltenberg II, 2008]. The protection prohibits cod capture with ships above 15 m within defined "fjord lines" and a prohibition against Danish seines in the same areas. Flatfish are primarily caught with Danish seines and traditionally as by-catch from coastal cod capture. Subsequently, since the introduction of the coastal cod protection, the general flatfish capture has steadily decreased, as shown in fig. 2.5 [Bjørklund and Henriksen, 2011, Ingolfsson et al., 2016]. The trend is apparent for plaice (*Pleuronectes platessa*), which had record-high capture levels of 3200 tonnes in 2003 to 794 tonnes in 2020, a decrease of 75% [Fiskeridirektoratet, 2022].

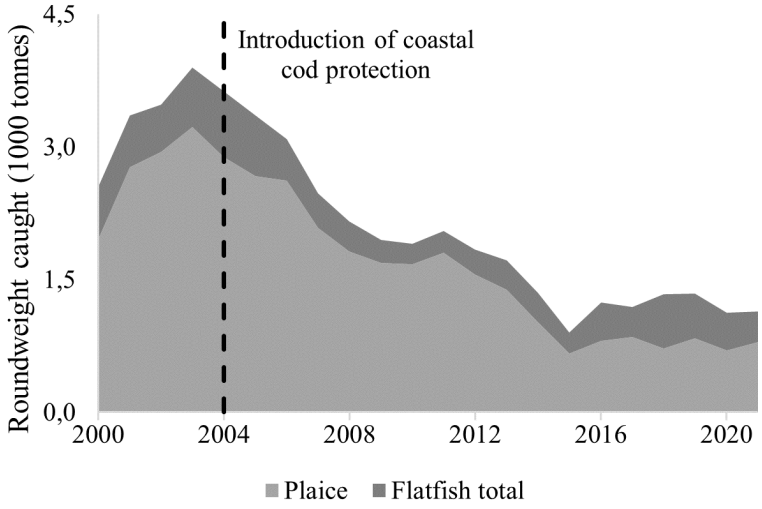

Figure 2.5: Yearly capture in round weight of total flatfish and plaice (*Pleuronected platessa*) in Norway before and after the introduction of the coastal cod protection. Data from Fiskeridirektoratet [2022].

The combined restrictions of Danish seines and bottom trawling make the capture method a bottleneck for flatfish capture. Increased catch amounts will only be possible if flatfish capture can be separated from coastal cod capture. Trials with a modified Danish seine showed promise of selectively catching flatfish [Ingolfsson et al., 2016].

2.2.3 Production and technology

Bottlenecks are also found in developing infrastructure and production methods to process the fish to obtain high quality and long freshness. Flatfish and skates are typically caught as by-catch, in low volumes, and with little continuity. This makes stability, quality, and quantity varying parameters [Bjørklund and Henriksen, 2011]. A potential solution to counter low delivered volumes and obtain better continuity is to keep live storage of flatfish. Flatfish have been shown to deal well with live storage and survive for extended periods [Bjørklund and Henriksen, 2011]. However, live storage has challenges related to distribution costs and animal welfare.

Fish products are perishable commodities. Increasing the shelf life of flatfish and skates contributes to increased stability in the food production chain and reduces food loss and waste. Several studies have researched how to increase the shelf life of flatfish. Storage methods and on-board handling have been investigated for megrim, where fish treated with ozonated ice water on board had an acceptable quality after 14 days [Pastoriza et al., 2008]. A part of the OPTiMAT project at NTNU is to investigate factors that affect the shelf life of plaice. Effects of different packaging methods are tested to determine which packaging methods produce the least spoilage organisms and ensure the most extended shelf life. Preliminary and unpublished results show that a lower temperature (around 0

ºC) has the most beneficial effect on the shelf life of the flatfish, much in correspondence with the study by Pastoriza et al. [2008].

The high supply uncertainty and the high perishability of flatfish and skates require a supply chain that is flexible with a high supply chain speed [Romsdal, 2014]. This means that scale production benefits are not obtained, and the production risk being resource-demanding and costly. Quantitative modeling of chain optimization can be applied to optimize costs and profit in the fisheries' supply chains [Jensen et al., 2010].

2.2.4 Marked and distribution

The small market of flatfish and skates in Norway is a bottleneck in higher exploitation of the fish. Efforts are needed in market development and the establishment of distribution channels, both in Norway and in foreign countries. There is a lack of infrastructure and knowledge about barriers to the distribution of flatfish in Norway today [Bjørklund and Henriksen, 2011]. The use of the same channels as halibut (*H. hippoglossus*), catfish (*Anarhichas* sp.), and monkfish (*Lophius* sp.) is a possible solution, as these species are also taken sporadically, but have established distribution channels [Bjørklund and Henriksen, 2011]. Many European countries already have a more established market for flatfish. Developing distribution channels for these markets can ensure sales while higher demand in Norwegian markets is being developed. However, establishing a foreign market takes time and patience, market knowledge, and venture capital [Bjørklund and Henriksen, 2011].

Increasing market prices for the fish also remains a challenge. Although flatfish such as lemon sole and megrim obtain high prices, the low quantities caught simultaneously reduce the potential profitability. The species may obtain higher prices with a more renowned name in the market. Previous examples of this are monkfish and catfish, which went from badly reputed fish to renowned delicacies in a few decades [Havforskningsinstituttet, 2014]. Other fish, including flounder and thornback ray are in lower demand and harder to sell. For these species, the low market prices are significant hurdles to achieving a profitable industry.

Flatfish and skates can benefit from several worldwide food trends in their marketing strategy. Two emerging and increasingly important food trends in the 21st century are sustainability and healthy food trends [Mellentin, 2022]. The sustainability trend can link the consumption of flatfish and skates to better utilization of the ocean's resources. Additionally, consumers are starting to demand sustainable harvesting of marine resources, which increases the potential of LUR species [Bjørklund and Henriksen, 2011]. Adherence to sustainability labels, such as Marine Stewardship Counsil (MSC), can be essential in this marketing strategy. Another critical trend is healthy foods, which includes highlighting the nutritional aspects of the fish.

2.3 Chemical and nutritional investigations of flatfish and skates

Few studies have been conducted on the nutritional composition of the investigated species. More knowledge on the nutritional quality of flatfish and skates can contribute to the development of commercial capture and increased consumption in Norway and globally.

2.3.1 Proximate composition of flatfish and skates

A few studies have considered the chemical composition of some flatfish species, including megrim. A summary of the previous findings on flatfish and thornback ray proximate composition is summed up in table 2.1. The carbohydrate content is not included, as the content is assumed low in fish.

The conducted studies indicate that flatfish, in general, are lean to low-fat fish species (∼0.5 to 1.9% lipid content) with a high water content (from 76% to 82%) [Afonso et al., 2013, Barbosa et al., 2018, Karl et al., 2013, Martínez et al., 2010, Ruff et al., 2002]. For the two studies found on thornback ray, the fat content varied markedly from 0.5% to 3.4% [Colakoglu et al., 2011, Turan et al., 2007]. In previous studies, flatfish and ray fillets seem to be good protein sources, with protein content varying between 16% and 22% [Afonso et al., 2013, Barbosa et al., 2018, Colakoglu et al., 2011, Karl et al., 2013, Martínez et al., 2010, Ruff et al., 2002, Turan et al., 2007]. However, several flatfish species have not been investigated in this regard, and little is known about their chemical composition and distribution.

Table 2.1: Proximate composition studies for different species of flatfish (Pleuronectiformes) and thornback ray (*Raja clavata*).

	Megrim[1,2,3] (*Lepidorhombus whiffiagonis*)	**Plaice**[4] (*Pleuronectes platessa*)	**Alaskan soles**[4] (*Lepidopsetta species*)	**Yellowfin sole**[4] (*Limanda aspera*)	**Turbot**[5,6,7] (*Scophthalamus maximus*)	**Halibut**[6] (*Hippoglossus hippoglossus*)	**Thornback ray**[8,9] (*Raja clavata*)
Water (%)	78–79	81.7	78–80	82.1	78–80	76.1	77
Ash (%)	1.2–1.4	0.9	1.1–1.5	1.0	1.0–1.2	0.9	1.1–1.4
Protein (%)	18–19	16.6	18–20	16.0	17–19	22.6	19–20
Lipid (%)	0.9–1.9	0.8	1.0	1.2	0.7–1.3	0.5	0.5–3.4

[1][Afonso et al., 2013] [2][Barbosa et al., 2018] [3][Pastoriza et al., 2008]
[4][Karl et al., 2013] [5][Martínez et al., 2010] [6][Ruff et al., 2002]
[7][Özogul et al., 2006] [8][Colakoglu et al., 2011] [9][Turan et al., 2007]

2.3.2 Marine proteins

Proteins consist of both indispensable and dispensable amino acids, the former being amino acids the human body cannot produce in sufficient quantities on its own [Coultate, 2016]. There are nine groups of indispensable amino acids (IAA), and their minimum intake requirements for humans set by FAO et al. (2007) are shown in table 2.2. Lysine, methionine, threonine, and tryptophan are among the most common limiting amino acids in foodstuffs, especially from plant-based protein sources [FAO et al., 2007, p. 136].

Table 2.2: Current estimates for indispensable amino acid intake levels for adults expressed as mg/kg body weight per day and mg/g protein [FAO et al., 2007, p.245].

Amino acid	mg/kg body weight per day	mg/g protein
Histidine	10	15
Isoleucine	20	30
Leucine	39	59
Lysine	30	45
Methionine+cysteine	15	22
Phenylalanine + tyrosine	25	38
Threonine	15	23
Tryptophan	4	6
Valine	26	39
Total indispensable amino acids	184	277

The assessment of a food protein source is a function of both the protein quantity and the protein quality [FAO et al., 2007, p. 93]. Marine muscle tissues typically contain a high amount of protein and are considered good protein sources. Previous investigations of flatfish have shown that the protein content ranges from 16% to 22%, while around 19% to 20% for thornback ray [Colakoglu et al., 2011, Karl et al., 2013, Martínez et al., 2010, Ruff et al., 2002].

The protein quality of a foodstuff can be determined by calculating the digestible indispensable amino acid score (DIAAS) [FAO, 2013]. The score is a product of the amino acid scoring pattern of the protein and the digestibility of these amino acids. The amino acid scoring pattern is related to how the amino acids in the protein correspond to the nutritional requirements set by FAO [2013]. A beneficial amino acid content is characterized by a high IAA content, which exceeds the nutritional requirements. Digestibility explains the degree the body digests the proteins and the amino acids absorbed and readily used in biological reactions. Like most animal proteins, marine proteins have an excellent digestibility, around 94% for whole protein sources [FAO et al., 2007, p. 96]. However, to obtain precise results for digestibility, FAO [2013] now recommends using specific ileal digestibility factors for each indispensable amino acid in different food systems.

The digestible IAA reference ratios can be calculated for each IAA from the amino acid content and the ileal digestibility. IAA ratios above one are characterized by a high con-

tent of the digestible indispensable amino acid, which exceeds nutritional recommendations. IAA ratios below one means that the digestible IAA in the protein does not meet the recommendations. The lowest IAA reference ratio is multiplied by 100 to obtain the DIAAS. Food with scores above 100 can be classified as "excellent" protein quality sources, scores between 75 and 100 can be classified as "good" protein quality sources, while scores below 75 are "low" protein quality sources [FAO, 2013]. Previous investigations on DIAAS in fish have determined them to be of excellent protein quality [Burd et al., 2019, Shaheen et al., 2016].

In addition to their nutritional importance, amino acids are associated with taste when they occur unbound in the form of free amino acids (FAA) in biological systems. FAAs have been recognized as essential taste contributors in seafood [Fuke and Konosu, 1991, Kirimura et al., 1969, Sarower et al., 2012]. Glutamic acid, glycine, and alanine are commonly identified among the most important taste contributors. Glycine and alanine are linked to sweetness, while FAAs such as valine, arginine, lysine, and methionine are linked to bitter taste in seafood [Sarower et al., 2012]. Aspartate and glutamate both provide a sour taste. However, they also give an umami taste in the presence of sodium salts, such as the familiar monosodium glutamate (MSG). Phenylalanine and tyrosine also have a bitter taste, but can enhance the umami flavor [Sarower et al., 2012]. The taste production is complex, with synergistic and antagonistic effects present between several taste compounds. The relative quantities among the flavor compounds and pH have also been shown to affect the perceived taste [Sarower et al., 2012]. Arginine has, for example, been hypothesized to have both a bitter taste with a hint of sweetness and to lack taste but function as a flavor potentiator contributing to fullness [Fuke and Konosu, 1991, Sarower et al., 2012].

Previous findings have found huge intraspecific variation for FAAs in four flatfish species, including lemon sole [Jones, 1959, Karl et al., 2013]. Taurine was found to be the most abundant FAA constituent in all the investigated four species, but also FAAs glycine, alanine, and lysine were commonly abundant. Karl et al. [2013] found some interspecific variations, where Northern rock soles contained higher levels of lysine while plaice had higher levels of histidine.

2.3.3 Marine lipids

Marine lipids have long been reputed for their health effects on humans, and this stems primarily from their fatty acid (FA) composition and distribution [Coultate, 2016, p. 113]. Marine lipids contain a high degree of unsaturated and longer-chain FAs [Coultate, 2016, p. 121]. Unsaturated FAs include both mono- and polyunsaturated fatty acids (MUFAs and PUFAs, respectively). Of investigated flatfish, up to 50% of FAs were PUFA, and only around 18% to 25% of the FAs saturated (SFA) [Afonso et al., 2013, Aubourg et al., 2007, Barbosa et al., 2018, Dwyer et al., 2003, Karl et al., 2013, Martínez et al., 2010]. For thornback ray, the proportion of SFAs was higher, measured between 39% and 48%, in studies conducted by Colakoglu et al. [2011] and Turan et al. [2007].

Marine lipids also consist of a high amount of FAs humans cannot produce themselves, namely n3 FAs [Coultate, 2016, p. 113]. Marine lipids are the primary source of n3 FAs docosahexaenoic acid (DHA, C22:6) and eicosapentaenoic acid (EPA, C20:5), which are reputed for their effects on reducing blood pressure, coronary heart disease, and inflammatory diseases [Coultate, 2016, Helsedirektoratet, 2011, Mozaffarian and Wu, 2012, pp. 88, 121]. European adults are therefore advised to eat 500 mg EPA+DHA daily [EFSA, 2012, Kris-Etherton et al., 2002]. The latter years, there has also been an increasing focus on docosapentaenoic acid (DPA, C22:5) for the same reasons as DHA and EPA [Mozaffarian and Wu, 2012]. In flatfish, DHA and EPA are among the most abundant FAs, making up more than 40% of total FAs for plaice (*P. platessa*) and yellowtail flounder (*Limanda ferruginea*) [Dwyer et al., 2003, Karl et al., 2013]. Afonso et al. [2013] found that a 160 g portion of megrim filet provided 84% of recommended daily EPA+DHA intake. While DHA is the most abundant n3 FA in some species (megrim, yellow-tail flounder, thornback ray), EPA is the most abundant in others (plaice, Alaskan soles, turbot). The n3 FA content appears lower for thornback ray, especially for EPA.

The rational intake of n3/n6 FAs in the human diet has been linked to the prevalence of coronary heart disease [Coultate, 2016, p. 121]. Humans in the Western world consume an n3/n6 ratio of around 1:15-20 while a recommended intake is 1:6 [Coultate, 2016, Wijendran and Hayes, 2004, p. 121]. For this reason, both the Norwegian Dietary advice and the Food and Agriculture Organization (FAO) recommend a higher level of fish intake [FAO, 2020, Helsedirektoratet, 2011, p. 88]. Flatfish and thornback ray appear to have a beneficial n3/n6 FA ratio, ranging from 4-14:1 for investigated species [Aubourg et al., 2007, Barbosa et al., 2018, Karl et al., 2013, Ruff et al., 2002]. Table 2.3 sums up previous investigations on FA distribution in flatfish and thornback ray.

Table 2.3: Fatty acid composition and n-3/n-6 ratio for muscles from various flatfish species (Pleuronectiformes) and thornback ray (*Raja clavata*). Data are modified from source.

		Megrim[1,2] (*Lepidorhombus whiffiagonis*)	**Four-spotted megrim**[1] (*Lepidorhombus boscii*)	**Plaice**[3] (*Pleuronectes platessa*)	**Alaskan soles**[3] (*Lepidopsetta* species)	**Turbot**[4,5] (*Scoptthalamus maximus*)	**Yellow-tail flounder**[6] (*Limanda ferruginea*)	**Thornback ray**[7,8] (*Raja clavata*)
Total lipids (% of ww)		0.9–1.6	0.7	0.8	1.0	0.7–1.3	1.3	3.4
Fatty acid composition (%)	SFA	18–22	20.2	25	25–26	25		39–48
	MUFA	22–31	20.2	25	22–29	8–27		13–18
	PUFA	23–25	25.4	51	46–52	50–66	36–50	24–43
	- 20:5 (EPA)	3.5–4.7	4.7	22	29–30	25–51	15	1.5–6
	- 22:6 (DHA)	13.4–17	12.4	19	11–18	8–10	31	12–15
n-3/n-6 ratio		6–11	4	5	10–13	8–14	8–13	4

[1][Afonso et al., 2013] [2][Barbosa et al., 2018] [3][Karl et al., 2013]
[4][Aubourg et al., 2007] [5][Martínez et al., 2010] [6][Dwyer et al., 2003]
[7][Colakoglu et al., 2011] [8][Turan et al., 2007]

2.3.4 Factors affecting the chemical composition

Several factors affect the biochemical composition of flatfish, including the type and location of fish muscle and season.

An inhomogeneous distribution of lipids has been shown within muscle tissue for several species. It is now widely recognized that dark muscle contains more lipids than white muscle, and differences between dorsal and ventral fillets for several fish species have been found [Body and Vlieg, 1989, Ingemansson et al., 1991, Testi et al., 2006]. In flatfish, lipid distribution has previously been examined for megrim and turbot, where a higher lipid proportion was found in edge zones than central fillets [Aubourg et al., 2007, Barbosa et al., 2018]. The same study from Barbosa et al. [2018] found that upper and lower fillets did not have different lipid contents in total lipids, types of lipids, or FAs. Furthermore, a study from the OPTiMAT project conducted on plaice found no differences between upper belly and lower loin fillets in terms of proximate composition, TAA, FAA and FA contents [Kendler, 2022, work in progress].

Seasonal variation has been shown to affect several biochemical aspects of seafood. For lipids, seasonal variation is found for numerous species, including herring (*Clupea harengu*), cod (*Gadus morhua*), and sprat (*Sprattus sprattus*), where lipid contents are highest in the Fall and lowest in Spring [Jangaard et al., 1967, Røjbek et al., 2013]. Unpublished results on the investigation of seasonal effects on the chemical composition of European plaice from Kendler [2022, work in progress] indicate that fish undergoes a change in proximal composition between September, December, and April, with higher levels in December

compared to April. Taste-active FAA contents also vary with season [Sarower et al., 2012]. In lemon sole, significant seasonal variations for FAAs glutamic acid and glycine were found, with the highest levels in February-March and the lowest in September-October [Jones, 1959].

Other factors that can affect the biochemical composition of flatfish and skates include the type of food and starvation, temperature and salinity of the water, and sex and gonad maturation [Jones, 1959]. Water temperature affects lipid composition, and previous studies have shown that same-species fish kept at colder temperatures have enhanced levels of MUFA and PUFA compared to fish kept at warmer temperatures [Tocher et al., 2004]. This mechanism is upregulated levels of enzymes that desaturate and elongate FAs to ensure cell membranes with correct fluidity at lower temperatures [Fadhlaoui and Couture, 2016]. The feed was thought to be the main reason for FA differences between wild and farmed turbot, where wild turbot had twice the amount of DHA compared to farmed fish [Martínez et al., 2010]. Additionally, the processing of the fish post-mortem affects the nutritional aspects of the fish. Smoked thornback ray was found to have a higher n3/n6 ratio than raw fish but lower total values of n3 [Colakoglu et al., 2011].

Chapter 3

Materials and methods

A flowchart of the experimental setup of the thesis is found in fig. 3.1.

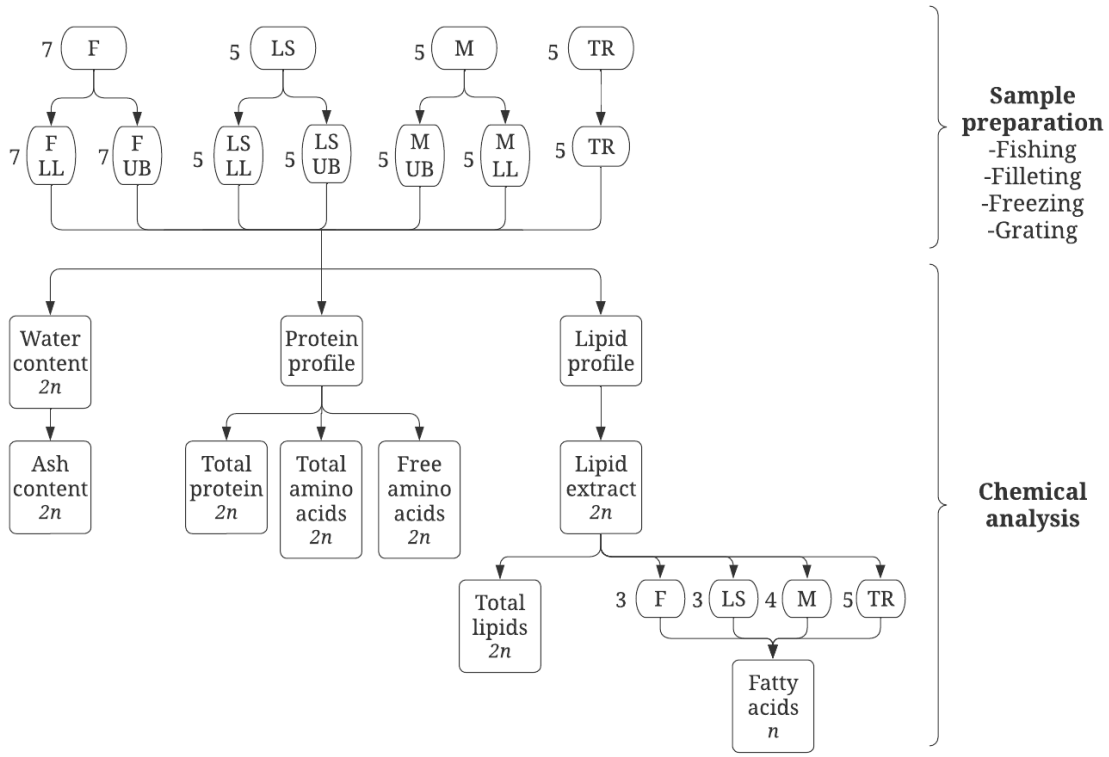

Figure 3.1: The experimental design of the master thesis. The experimental phase consisted of one sample preparation phase and one chemical analysis phase. Samples were prepared from flounder (F, *Platichthys flesus*), lemon sole (LS, *Microstomus kitt*), megrim (M, *Lepidorhombus whiffiagonis*), and thornback ray (TR, *Raja clavata*), and with lower loin (LL) and upper belly (UB) fillets kept from the flatfish species. All analyses were performed in duplicates (2n) on each fillet, except for fatty acid analysis (n).

3.1 Sample preparation

Fish were caught on the Norwegian west coast in September 2020 and April 2021. Sample specifications are shown in table 3.1.

Table 3.1: Sample specifications for flounder (*Platichthys flesus*), lemon sole (*Microstomus kitt*), megrim (*Lepidorhombus whiffiagonis*), and thornback ray (*Raja clavata*).

Season	**September**	**April**
Coordinates	62.7356 N. 6.4889 E 69.7292 N. 6.3675 E	62.7356 N. 6.4889 E 62.8133 N. 6.6105 E
Water temperature (°C)	13.0	5.4
Samples	Flounder n=5 Lemon sole n=2 Megrim n=5 Thornback ray n=5	Flounder n=2 Lemon sole n=3

A total of seven flounders, five lemon soles, five megrim, and five thornback rays were caught. Immediately after capture, the fish were gutted, then kept on ice until the end of rigor mortis. Fish were either filleted directly or frozen (-80 °C) and subsequently thawed before filleting. Two fillets from each flatfish were used for further analysis, the lower loin (LL) and upper belly (UB), see fig. 3.2. One fillet was kept for thornback ray, which consists of only two fillets. All fillets were frozen directly and stored at -80 °C until use.

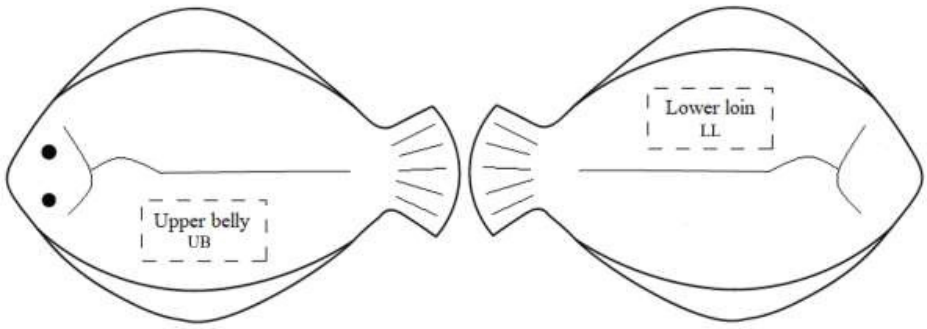

Figure 3.2: Upper belly (UB) and lower loin (LL) fillets used as samples for flounder (*Platichthys flesus*), lemon sole (*Microstomus kitt*), and megrim (*Lepidorhombus whiffiagonis*).

Before analyses, frozen fillets were grated manually to provide a homogenous sample. When preparing the sample, mainly the middle part of the fillets was grated, avoiding edge zones, skin, and bones, as shown in fig. 3.2 with dashed lines. Grated material was stored at -80 °C until further use.

3.2 Chemical analyses

3.2.1 Dry matter and ash content

Dry matter and ash content were determined following the AOAC 925.10 method [AOAC, 1990]. Frozen grated samples (1.15 ± 0.15 g) were weighed in duplicates in dried-out crucibles. The samples were placed in a dehydrator at 105 °C for 24 hours (Termaks, TS8056, Bergen, Norway). After 24 hours, the samples were placed in a desiccator to cool down to room temperature and weighed. The water content was calculated using the formula in eq. (3.1).

$$\text{Dry matter (g)} = \text{dry matter+crucible}(g) - \text{crucible}(g) \tag{3.1}$$

The dried samples were transferred to an ash oven and burned at 550 °C for 20 hours (Nabertherm, B410, Lilienthal, Germany). The samples were placed in a desiccator to cool down and weighed. The ash content was calculated using formulas in eq. (3.2).

$$\text{Ash (g)} = \text{ash+crucible}(g) - \text{crucible}(g) \tag{3.2}$$

3.2.2 Protein content by Kjeldahl method

Protein content was determined using the Kjeldahl method [AOAC, 1990]. Frozen grated samples (1.2 ± 0.3 g) were weighed out in duplicate on a filter paper (WhatmanTM, Maidstone, Great Britain) and placed in a 20 sample Kjeldahl rack. Two blanks (only filter paper) and two reference samples (glycine, 0.2 ± 0.1 g, CAS 54-40-6, Merck Millipore, Darmstadt, Germany) were used as controls. Sulfuric acid (15 mL, H_2SO_4, 95-97%, CAS 7664-93-9, Merck Millipore, Darmstadt, Germany) was added to the samples for protein hydrolysis, along with two Kjeldahl Tablets Eco (3.998 g K_2SO_4, 0.002 g $CuSO_4$-5 H_2O, Büchi, Flawil, Switzerland) to increase the boiling point of sulfuric acid and catalyze the reaction. In three steps, the samples were digested in a KjelDigester K-449 connected to Scrubber K-415 (Büchi, Flawil, Switzerland). The samples were heated to 280 °C, ran at 320 °C for 20 minutes, and at 420 °C for 90 minutes before cooling down to room temperature (35 minutes). The increased temperatures and the concentrated sulfuric acid oxidized the organic matter, liberating nitrogen as ammonium sulfate ($(NH_4)_2SO_4$).

Digested samples were titrated in the following steps using a KjelMaster system K-375 (Büchi, Flawil, Switzerland). First, water (H_2O, 25-90 mL) was added to dilute the acidic solution. NaOH (32%, 15-90 mL, CAS 1310-73-2, Merck Millipore, Darmstadt, Germany) was added to neutralize the nitrogen, converting NH_4 to gaseous NH_3. Distilled

NH_3 was collected by binding to a borate complex (addition of H_2BO_3, pH 6.65, 40-70 mL, CAS 10043-35-3, Merck Millipore, Darmstadt Germany) and titrated to determine the amount of nitrogen in the sample (pK_a=9.24). H_2SO_4 (0.25 mol/L) was used as the titration solution. Protein content was calculated using eq. (3.3), using a nitrogen conversion factor of 6.25 [NMKL, 2003].

$$\% \text{ N} = PF \cdot \frac{(V(1) - V(B1)) \cdot F \cdot c \cdot M(N) \cdot 100\%}{m} \tag{3.3}$$

Where:

- V(1): consumption of titrant, sample (L)
- V(B1): average consumption of titrant, blank (L)
- F: molar reaction factor for H_2SO_4
- c: concentration of titrant
- M(N): molecular weight of nitrogen (14.007 g/mol)
- m: weight of the sample (g)
- PF: protein factor (6.25 for fish, set by NMKL [2003])

3.2.3 Free and total amino acid content by high-pressure liquid chromatography

Total amino acids (TAA) were extracted from the samples following the method of Blackburn [1978]. Samples were freeze-dried as a preparation for the analysis. A total amount of 1.25 ± 0.50 g of sample was weighed and freeze-dried for 22 hours at < -40 °C and < 13.3 Pa (Labconco, FreeZone 12, Kansas, USA). Freeze-dried samples were stored at -40 °C.

Freeze-dried samples (80 ± 10 mg) were weighed up in duplicates, and 1 mL of HCl (6 M, CAS 7647-01-0, VWR International, Radnor, USA) was added. The tubes were incubated for 22 hours at 105 °C to allow protein hydrolysis. Screws were untied 30 minutes into the incubation to prevent pressure buildup. Hydrolyzed samples were pH-neutralized by adding drops of NaOH in different concentrations (6 M, 1 M, 0.1 M, CAS 1310-73-2, Merck Millipore, Darmstadt, Germany) to reach a pH between 6.5 and 7.5. The samples were filtered through a glass microfibre filter GF/C using suction (Whatman™, Maidstone, Great Britain) and subsequently filled up to 10 mL with deionized water to guarantee an equal volume of all samples. The samples were diluted in a 1:500 dilution with deionized water. Diluted samples were filtered through 0.22 μm polyethersulfone filters (Whatman™, Maidstone, Great Britain). Finally, 0.205 mL were pipetted into HPLC vials and stored at -40 °C before further analysis.

FAAs were extracted from the samples following the method of Osnes and Mohr [1985]. A total of 2.0 ± 0.2 g of frozen grated sample was placed into centrifuge tubes. Deionized water (10 mL) was added to the tubes, and the mixture was homogenized for 45 seconds at a speed of 9800 rpm to disrupt cells and release proteins (Ika, Ultra Turrax T25, Staufen, Germany). The tubes were centrifuged for three minutes at 7000 rpm at 4 °C to obtain two phases (Kubota, Model 1700, Tokyo, Japan). The soluble protein extract phase was taken out, and 1 mL of the extract was mixed with 0.25 mL of sulphosalicylic acid (10%, $C_7H_6O_6S$, CAS 97-05-2, Merck Millipore, Darmstadt, Germany) to allow protein breakdown. The samples were shaken for one minute at 1500 rpm at 4 °C using a thermoshaker (VWR International, Thermal Shake lite, Radnor, USA). The tubes were placed in a fridge (4 °C) for 30 minutes to guarantee the total breakdown of proteins. After protein breakdown, the tubes were centrifuged for 10 minutes at 10 000 rpm and 4 °C (ThermoFisher, Megafuge 8R Centrifuge, Waltham, USA). The supernatant (containing the FAAs) was diluted to 1:25 using deionized water. In duplicates, the diluted samples were filtered through 0.2 μm polyethersulfone membrane filters (Whatman™, Maidstone, Great Britain) to remove any protein trace. Finally, 0.205 mL of the samples were transferred to vials and stored at -40 °C before further analysis.

Both TAA and FAA were analyzed by high-performance liquid chromatography (HPLC). The solvent (methanol (CH3OH, CAS 67-56-1, VWR International, Radnor, USA) and sodium acetate (0.08 M, CAS 127-09-3, Alfa Aesar, Haverhill, USA) with 2% tetrahydrofuran (CAS 109-99-9, Merck Millipore, Damstadt, Germany) was run through a TSP P400 pump to ensure a high pressure and constant stream to the injection valve (ultimate 3000WP injector, Waters Corp., Milford, USA). At the injection valve, the sample was provided to the solvent and passed to a Nova-Pak C18 column (WAT086344, particle size: 4 μm, 3.9 mm*150 mm, Waters Corp., Milford, USA). The stationary phase was silica, and the amino acids were retarded and hence separated at different times along the stationary phase. The flow rate was adjusted to 0.9 mL/min. After passing through the column, the amino acids were detected by fluorescence and recognized by a Dionex RF2000 detector (ThermoFisher, Waltham, USA). Alpha-aminobutyric acid was used as an internal standard. Three amino acids were not analyzed: cysteine, proline, and tryptophan. The amino acids glycine and arginine were co-eluted in the analysis. Amino acid amounts were calculated using eq. (3.4).

$$\frac{\text{mg amino acid (AA)}}{\text{g fish}} = \frac{M(AA) \cdot c \cdot d}{m \cdot 10^6} \tag{3.4}$$

Where:

- M(AA): molecular weight of amino acids (g/mol)
- c: molecular concentration of amino acids in the sample (nmol/mL)
- d: dilution factor (for FAAs $25 \cdot 10 \cdot 1.25$, for TAA $10 \cdot 500$)

– m: weight of the sample (g)
– 10^6: conversion factor from μg to g

For TAAs, mg AA per g protein was calculated using eq. (3.5a), and amino acid scores were calculated using eq. (3.5b) as proposed in FAO et al. (2007, p. 95):

$$\text{mg AA per g protein} = \frac{\text{mg AA per g wet weight}}{\text{g protein per g wet weight}} \cdot 100 \tag{3.5a}$$

$$\text{Digestible IAA reference ratio} = \frac{\text{mg of AA in 1 g test protein} \cdot \text{df}}{\text{mg of AA in 1 g of reference protein}} \tag{3.5b}$$

Where:

– df: True ileal digestibility factor for specific amino acids in fish as proposed by FAO (2012) and shown in Appendix A.
– Reference protein: nutritional requirements set by FAO et al. (2007).

The DIAAS was calculated as shown in eq. (3.6) as proposed in FAO (2013).

$$\text{DIAAS (\%)} = \text{lowest value of DIAA reference ratio} \cdot 100 \tag{3.6}$$

3.2.4 Lipid content by Bligh and Dyer

The lipid content of the samples was determined following the method of Bligh and Dyer [1959]. Samples (1.2 ± 0.1 g) were weighed up in duplicates, and 4 mL of deionized water, 10 mL of methanol (CH_3OH, CAS 67-56-1, VWR International, Radnor, USA), and 5 mL of chloroform ($CHCl_3$, CAS 67-66-3, VWR International, Radnor, USA) were added to the samples. The samples were homogenized for 60 seconds at 9800 rpm to disrupt cells and release lipids (Ika, Ultra Turrax T25, Staufen, Germany). Chloroform (5 mL, CAS 67-66-3, VWR International, Radnor, USA) was added, and the samples were homogenized for 20 seconds to solubilize the lipids in the chloroform. Deionized water (4 mL) was added to the samples, and the samples were homogenized for 20 seconds. The samples were kept on ice during homogenization to avoid heating up. The samples were centrifuged at 7000 rpm for 10 minutes at 3 °C (Kubota, Model 1700, Tokyo, Japan). After the centrifugation, the chloroform phase containing the lipids was pipetted into a

separate beaker. The chloroform phase (2 mL) was pipetted in duplicates into preweighed tubes. The chloroform solvent was evaporated under a nitrogen stream for 15 minutes at 60 °C. The remaining extracted lipids were weighed, and the lipid content was calculated using eq. (3.7).

$$\text{Lipid content (\%)} = \frac{\text{(Tube with extracted lipid (g)-empty tube (g))} \cdot V_{CHCl_3 \text{ added}}}{\text{Original sample (g)} \cdot V_{CHCl_3 \text{ taken out}}} \quad (3.7)$$

The remaining chloroform phase containing lipids was frozen and stored at -80 °C to use for FA analysis.

3.2.5 Fatty acid distribution by gas chromatography

FAs were prepared as methyl esters for analysis by gas chromatography (GC). For fatty acid methyl ester (FAME) preparation, the method of Metcalfe et al. [1966] was used. Chloroform phases containing lipids from individual fish were systematically merged to obtain three samples each of flounder and lemon sole, four samples of megrim and five samples of thornback ray. Merging samples was done to obtain at least 0.02 g of lipids per sample. Nitrogen evaporation was conducted at 30 °C until all chloroform was removed from the samples, and 3 mL NOH in methanol (0.5 M, CAS 67-56-1, VWR International, Radnor, USA) was added to the samples and vortexed to saponify the lipids. Samples were incubated in a water bath at 70 °C for 20 minutes, vortexed, and cooled on ice. Afterward, 5 mL of boron trifluoride-methanol (14%, BF_3, CAS 7637-07-2, Merck Millipore, Damstadt, Germany) was added to allow acid-catalyzed esterification of the FAs. The samples were re-incubated in the water bath at 70 °C for five minutes and cooled on ice. N-butyl acetate (2 mL, CAS 123-86-4, Merck Millipore, Damstadt, Germany) was added, and the samples were shaken carefully by turning the tubes five times each. Saturated NaCl (around 1.5 mL, CAS 7647-14-5, VWR International, Radnor, USA) was added to the samples until the liquid was one cm from the top of the tube to better separate the phases. Two spatulas of powdered sodium sulfate (Na_2SO_4, CAS 7757-82-6, VWR International, Radnor, USA) were added, and the samples were rested at room temperature (21 °C) for five minutes to allow phase separation. Around 0.5 mL of hexane (CAS 110-54,3, VWR International, Radnor, USA) was added to the samples to visualize the lipid phase better. The lipid phase was then pipetted out and filtered through a 0.2 μm PTFE membrane (WWR International, Radnor, USA) into vials.

The FAMEs were analyzed by a GC apparatus (Agilent 6850, Agilent Technologies, Santa Clara, USA). The samples (2-3 μL) were introduced by an evaporation injector (inlet: 260 °C, pressure: 18.1 psi). Hydrogen was used as a carrier gas to pass the samples onto a polyethylene glycol column (HP-INNOWAX, i.D.: 0.25mm; film: 0.25μm, Agilent Technologies, Santa Clara, USA), where FAMEs were separated at different times along

the stationary phase. A flame ionization detector (FID, Agilent Technologies, Santa Clara, USA) adjusted to 310 °C was used to detect the samples. The oven program was set to a constant temperature of 160 °C for three minutes, with an increase of 3 °C/min to 240 °C, and held for three minutes.

FAs were identified by comparing retention times with a rapeseed standard (MilliporeSigma, Saint-Louis, USA) and a 37 component FAME mix (MilliporeSigma, Saint-Louis, USA). For a given FA i, relative quantification was performed using eq. (3.8).

$$\% \text{ fatty acid}_i = \frac{\text{Area}_{\text{fatty acid } i}}{\text{Area}_{\text{total fatty acids}}} \tag{3.8}$$

An estimation of the total amounts of FAs per 100 g edible fillet of fish was conducted using eq. (3.9).

$$\text{g FA per 100 g ww fillets} = \text{weight-\% FAME} \cdot \text{FACF} \cdot \text{lipid content} \tag{3.9}$$

Where:

- Weight-% FAME: from FAME analysis, assumed the same as weight%-FA since the marine lipids mainly consisted of long-chain FAs (Weihrauch et al., 1977).
- FACF: Fatty acid conversion factor (g FA/g lipid): from conversion factors proposed by Weihrauch et al. (1977) and shown in appendix B.
- Lipid content (in g lipid per g ww fillet): from Bligh and Dyer method

3.3 Data analysis

All data analysis and calculations were performed in Microsoft Excel (version 2202, Microsoft Corp, Redmond, USA). For flatfish representatives, analyses were performed in quadruplicates for species calculations for proximate composition, TAA and FAA, and duplicates for fillet calculations. For thornback ray, the same analyses were performed in duplicates. For FA distribution, the analysis was only performed in singlicate for all species due to the low amount of sample material. All statistical analyses were performed in SPSS Statistics (version 26, IBM Corp, New York, USA). Data were analyzed using one-way analysis of variance (ANOVA) with Tukey's posthoc tests, where statistical differences were found (α=0.05).

Chapter 4

Results and discussion

The biochemical composition of flounder (*P. flesus*), lemon sole (*M. kitt*), megrim (*L. whiffiagonis*), and thornback ray (*R. clavata*) was investigated to increase knowledge of the species. The proximate composition, protein, and lipid profile were found, and the nutritional value of the species was determined.

In addition to species comparison, differences between upper belly and lower loin flatfish fillets were investigated for proximate composition, TAA and FAAs. Upper belly fillets did not significantly differ ($P > 0.05$) from lower loin fillets for any of the species. Hence, these results are not fully presented but are available in appendix C. The findings correspond with a previous study in the OPTiMAT project on plaice, where no difference between the muscle samples from upper and lower body fillets was found for proximate composition, TAA, FAA, or FA [Kendler, 2022, work in progress]. For the megrim, the results were supported by the study of Barbosa et al. [2018], which found no differences in lipid content between upper and lower fillets. The upper and lower fillets thus had the same proximate, TAA, and FAA composition for the flounder, the lemon sole, and the megrim. Fillet differences for the thornback ray were not investigated as it only contains two fillets.

4.1 Proximate composition

To get basic information on the chemical composition of flounder, lemon sole, megrim, and thornback ray, the proximate composition was analyzed, including the water, ash, protein, and lipid content. The results of their proximate composition are shown in table 4.1. The species had a comparable chemical composition, with the water content ranging from 78% to 83% ww, the ash content from 0.8% to 1.2% ww, the protein content from 17% to 24% ww, and the lipid content from 0.5% to 1.2% ww.

Table 4.1: Proximate composition for central fillets of flounder (*Platichthys flesus*, n=7), lemon sole (*Microstomus kitt*, n=5), megrim (*Lepidorhombus whiffiagonis*, n=5), and thornback ray (*Raja clavata*, n=5).

Proximate composition* **(% of wet weight)**	**Flounder**	**Lemon sole**	**Megrim**	**Thornback ray**	**P-value**
Water (%)	82.1 ± 1.2^{bc}	84.4 ± 1.0^{c}	79.2 ± 1.4^{a}	80.1 ± 0.8^{ab}	0.001
Ash (%)	1.1 ± 0.1^{b}	1.0 ± 0.1^{ab}	1.1 ± 0.1^{b}	0.9 ± 0.1^{a}	0.003
Protein (%)	16.9 ± 1.0^{a}	17.5 ± 0.6^{a}	19.6 ± 1.1^{b}	24.0 ± 1.4^{c}	< 0.001
Lipid (%)	0.9 ± 0.1^{bc}	0.7 ± 0.2^{a}	1.0 ± 0.2^{c}	0.8 ± 0.1^{ab}	0.008
Total	101.1 ± 1.6	100.7 ± 1.3	100.9 ± 1.8	105.7 ± 1.6	

*Superscript letters represent significance between species (α = 0.05).

The megrim was found to have the lowest average water content of 79.2%, being significantly lower than the lemon sole and the flounder ($P < 0.001$). The measured water content for the megrim matched that found by Afonso et al. [2013], and Barbosa et al. [2018], who found values from 78% to 79%. The thornback ray was found to have a lower water content than the lemon sole, although the measured water content of 80% was a few percentage points (pp) higher than previous investigations by Colakoglu et al. [2011] and Turan et al. [2007] of 77%. The lemon sole and the flounder had the highest average water contents at 81% to 82%. For these species, the water content was around the two (81.7% and 82.1%) found for plaice and yellowfin sole by Karl et al. [2013].

The ash content of the three flatfish representatives was from 0.9% to 1.2%, while the thornback ray had slightly lower values than the flounder and the megrim with a range from 0.7% to 0.9% ($P = 0.003$). This was in line with previous investigations on flatfish, although the previous investigations on thornback ray found slightly higher ash values (from 1.1% to 1.4%) for this species [Colakoglu et al., 2011, Turan et al., 2007].

For protein content, the differences were more significant between the species. The megrim had a significantly higher protein content than the lemon sole and the flounder, with an average of 19.6% ($P < 0.001$), being one pp higher than the studies by Afonso et al. [2013] and Barbosa et al. [2018]. The lemon sole and the flounder had protein contents from 16% to 18%, in line with previous investigations of plaice (16.6%) and yellowfin sole (16.0%) of the same family [Karl et al., 2013].

The thornback ray had the highest average protein content of 24.0%, significantly higher than the flatfish species ($P < 0.001$). The measured protein content was not in correspondence with previous studies and was four to five pp higher [Colakoglu et al., 2011, Turan et al., 2007]. However, the standard deviation was low, with a fractional uncertainty of 5.8%, and there was good agreement between parallels. This points toward a systematic error in the method or true variance in the protein content of the fish. The previous studies

were conducted on thornback ray landed in Turkey, where living conditions for the fish are different from that of the Norwegian sea. Additionally, the Kjeldahl method measures nitrogen content based on the average 16% nitrogen content in proteins. However, other interfering substances in the cells also contain nitrogen, leading to an overestimation of the protein nitrogen in the food [Mæhre et al., 2018]. Skate tissue contains around 350 to 400 mM urea, which might have been an interfering substance in the Kjeldahl analysis [Wright, 2011].

The lipid content of the megrim was the highest among the investigated species, with a range from 0.8% to 1.2% ww, and its lipid content was significantly higher than that of the lemon sole and the thornback ray ($P = 0.008$). The findings were on the lower range of lipid content found for megrim in previous investigations, with a study by Pastoriza et al. [2008] finding a lipid content of up to 1.9%. Based on previous findings and this study, megrim can be classified as a low-fat species. The lemon sole had the lowest lipid content among the species with an average of 0.7% ww, significantly lower than the megrim and the flounder ($P = 0.008$). The flounder had a lipid content range of 0.8% to 1.0%, and both species can be classified as lean fish in line with other investigations on flatfish species [Karl et al., 2013]. The standard deviations for lipid contents were high, with the lemon sole and the megrim having a fractional uncertainty of 22.1% and 22.0%, respectively.

For the thornback ray, the measured lipid content was in the range from 0.7% to 0.9% ww. This was slightly higher than the finding of Turan et al. [2007] of 0.5% ww, while much lower than the finding of Colakoglu et al. [2011] of 3.4% ww. Two previous studies on deep-sea fish found that general deep-sea elasmobranchs had a lipid content of around 0.7% to 1.0%, and the current finding agrees with those studies [Synnes et al., 2007, Økland et al., 2005]. This present study supports that thornback ray is a lean fish as opposed to being a high-fat fish as Colakoglu et al. [2011] determined.

The sum of the proximate composition, being the total composition, equates to 100%. As so, this value gives information on the accuracy of the experimental procedures. The calculated sum of the proximate composition with standard deviation is shown in table 4.1. For the thornback ray, the calculated sum range from 104.1% to 107.3% was an indication that some of the proximate composition findings were too high. This could be linked to the high protein content found for the species, which did not match results from previous investigations of 18% to 19% by Turan et al. [2007] and Colakoglu et al. [2011]. This high sum range supports that the Kjeldahl analysis overestimated the protein content, with urea as a possible interfering substance [Mæhre et al., 2018].

Overall, the findings of this study are supported by previous investigations. However,some differences between studies were found for all species, which could be explained by various external factors, such as seasonal variation, sex, maturity, and feeding habits [Jones, 1959]. Most fish in this study were caught in September, when lipid contents are often higher than in Spring after the spawning season. A few samples were caught in April (for the lemon sole and the flounder), where lipid contents are often lower [Jangaard et al., 1967, Røjbek et al., 2013].

4.2 Total amino acids

TAAs were investigated to determine the nutritional value of the proteins in the fish. The TAAs results for the species are found in table 4.2. The most abundant TAAs for all species were leucine and lysine, as well as glutamic and aspartic acid. The same abundant TAAs were found in an investigation of three flatfish species by Kim and Lall [2000], although they found higher amounts of glycine. The glutamic and aspartic acid contents were overestimated, as glutamine and asparagine were converted to these two amino acids during acid analysis [Holt et al., 1971]. Consequently, asparagine and glutamine were detected in the lowest amounts in all samples.

Table 4.2: Total amino acid contents for central fillets of flounder (*Platichthys flesus*, n=7), lemon sole (*Microstomus kitt*, n=5), megrim (*Lepidorhombus whiffiagonis*, n=5), and thornback ray (*Raja clavata*, n=5).

	Total amino acids (TAA) content (mg/g wet weight)*				
Amino acid	**Flounder**	**Lemon sole**	**Megrim**	**Thornback ray**	**P-value**
His	2.7 ± 0.4^{a}	3.1 ± 0.4^{ab}	3.6 ± 0.7^{b}	2.5 ± 0.2^{a}	0.006
Ile	5.7 ± 0.6^{a}	4.7 ± 0.4^{a}	7.1 ± 0.5^{b}	5.7 ± 0.5^{a}	0.001
Leu	11.3 ± 1.2^{a}	10.6 ± 0.9^{a}	13.2 ± 0.9^{b}	10.4 ± 1.0^{a}	0.002
Lys	13.5 ± 1.6^{a}	13.7 ± 1.4^{a}	16.3 ± 0.9^{b}	12.1 ± 1.2^{a}	0.001
Met	4.6 ± 0.5^{b}	2.0 ± 0.4^{a}	4.2 ± 1.2^{b}	4.1 ± 0.4^{b}	< 0.001
Phe	5.6 ± 0.6	6.1 ± 1.6	6.8 ± 0.5	5.5 ± 0.5	-
Tyr	5.2 ± 0.6^{ab}	6.8 ± 4.2^{ab}	5.6 ± 1.0^{b}	4.1 ± 0.4^{a}	0.05
Thr	6.6 ± 0.8^{a}	7.0 ± 1.0^{a}	8.5 ± 0.4^{b}	6.5 ± 0.8^{a}	0.003
Val	6.3 ± 0.7^{ab}	5.8 ± 0.7^{a}	7.2 ± 0.9^{b}	5.6 ± 0.5^{a}	0.012
Sum IAA**	61.4 ± 6.9^{a}	60.7 ± 7.8^{a}	72.4 ± 4.1^{b}	56.5 ± 5.4^{a}	0.006
Asp	13.9 ± 1.5^{a}	13.6 ± 1.5^{a}	16.1 ± 1.3^{b}	12.6 ± 1.2^{a}	0.007
Glu	17.9 ± 1.9^{a}	18.3 ± 2.0^{a}	21.0 ± 1.5^{b}	16.8 ± 1.6^{a}	0.01
Asn	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	-
Ser	7.4 ± 1.2^{ab}	8.5 ± 2.0^{b}	8.4 ± 1.4^{b}	5.9 ± 0.7^{a}	0.01
Gln	0.3 ± 0.1^{ab}	0.1 ± 0.0^{a}	1.0 ± 1.1^{b}	0.4 ± 0.1^{ab}	0.05
Gly/Arg	7.9 ± 0.8^{ab}	8.3 ± 1.1^{ab}	9.0 ± 0.8^{b}	7.4 ± 0.8^{a}	0.05
Ala	8.9 ± 1.0^{ab}	7.4 ± 1.6^{a}	10.0 ± 1.5^{b}	7.5 ± 0.8^{a}	0.01
Sum non-IAA**	56.4 ± 6.2^{a}	56.4 ± 5.6^{a}	65.8 ± 5.3^{b}	50.8 ± 4.9^{a}	0.005
Total	117.8 ± 13.0^{a}	117.1 ± 13.3^{a}	138.1 ± 9.2^{b}	107.2 ± 10.3^{a}	0.005

*Superscript letters represent significant differences between species (α=0.05).

**Indispensible amino acids

The megrim generally contained a higher level of most amino acids. It had significantly higher levels of 12 of 16 investigated amino acids compared to the thornback ray, 10 of 16 compared to the lemon sole, and 7 of 16 compared to the flounder. The findings reflected the overall TAA content, where the megrim contained higher total levels than the other species ($P = 0.005$). The trend was also evident for indispensable amino acids (IAA), where the megrim contained significantly higher isoleucine, leucine, lysine, and threonine levels than all the other species.

Besides the megrim, there were fewer differences between the three other species. The thornback ray contained on average lower levels of most amino acids compared to the flounder and the lemon sole, but was only significantly lower than the lemon sole in its serine content ($P = 0.01$). The lemon sole distinguished itself with notably lower methionine levels than all the other species with an average of 2.0 mg/g ww, around half of the average 4 mg/g ww for the others. Apart from the methionine content, the lemon sole and the flounder did not differ in any TAA contents. Overall, the species thus displayed a similar TAA composition, with the same amino acids dominating in the different species.

The variance of the TAA results was generally low, with a fractional uncertainty ranging from 6.7% to 11.3% in total TAA contents. The lemon sole had the most considerable average variance, and the megrim had the lowest despite the highest contents. However, the contents of some amino acids had large standard deviations, notably for tyrosine in the lemon sole with a fractional uncertainty of 63% (6.8 $\pm$ 4.8 mg/g ww) and methionine in the megrim with a fractional uncertainty of 29% (4.2 $\pm$ 1.2 mg/g ww). There was generally good agreement between parallels which point toward a natural intraspecific variance.

In total, the megrim contained on average 13.8% (ww) total TAAs. This was 5.8 pp lower than the total protein results from the Kjeldahl analysis, where an average of 19.6% was found. The flounder and the lemon sole had TAA averages of 11.8% and 11.7%, respectively, which were 5.1 pp and 5.8 pp lower than the respective findings from the Kjeldahl method. As in the Kjeldahl method, the megrim contained significantly higher total protein amounts than the flounder and the lemon sole. The deviance can partly be caused by some amino acids getting decomposed during acid analysis, hence leading to lower results in the TAA analysis. Furthermore, cysteine, tryptophan, proline, and taurine were not measured in the analysis, which affects the total sum of amino acids. In the study by Kim and Lall [2000], these amino acids made up from seven to eight percent of all TAAs for the flatfish species. If this was the same for the species of this study, the species would all have one pp more TAAs. Moreover, a comprehensive study evaluating the differences in protein determination methods by Mæhre et al. [2018] states that the Kjeldahl method tends to overestimate the amount of protein in the sample, as the conversion factor of 6.25 has been shown to overestimate protein content generally. Therefore, the five to six pp deviance between the measured protein content in the Kjeldahl and TAA analyses can be regarded as a discrepancy between the two analytical methods. The actual protein content lies somewhere in between the findings of the Kjeldahl and TAA analyses.

For the thornback ray, the found TAA content of 10.7% was 13.4 pp lower than the finding from the Kjeldahl method. Furthermore, the results of the two analyses were opposite compared to the other species: In Kjeldahl, the thornback ray was found to contain the highest average protein content, while it had the lowest average protein content in TAA (not significant). The significant difference of 13.7 pp between analyses can not only be explained by some amino acid loss during sample preparation. The findings from the TAA method thus support the hypothesis that the results from the Kjeldahl analysis overestimated the protein content. If the thornback ray follows the same trend of a 5-6 pp deviance between the two protein determination methods as the flatfish species, thornback ray should contain around 14% to 18% protein. This would be even lower than previous investigations on the fish of 18% to 19% [Colakoglu et al., 2011, Turan et al., 2007]. Again, the interfering urea substance may explain this, as the previous investigations determined protein content using the Kjeldahl method.

The present thesis used the Kjeldahl analysis as the reference method for protein content as it is the official method for food protein determination by AOAC International [Latimer, 2016]. It was used in all previous proximate composition studies on flatfish and thornback ray, allowing for a more direct protein comparison. The Kjeldahl method is also a quick and straightforward procedure, and is cheaper with fewer equipment requirements than the TAA analysis. The TAA analysis was primarily conducted to obtain the amino acid distribution for the species, including IAA levels. However, Mæhre et al. [2018] concluded that the TAA method provides more precise total protein results than the Kjeldahl method.

The DIAAS for the species were calculated to determine the quality of the proteins, following the calculation proposed by FAO [2013]. The DIAAS scores are calculated based on the digestible IAA ratios. The digestible IAA reference ratios are displayed in table 4.3, and indicates whether all digestible IAAs were present in the protein in adequate amounts to meet the requirements for adults set by FAO et al. [2007]. To calculate the scores, the measured TAA levels were converted to mg/g protein for the different species, where TAA content was used for the conversion as the total protein indicator. Scores above one indicate sufficient IAA levels, while scores below one indicate insufficient levels.

Table 4.3: Total digestible indispensable amino acid ratios and scores (DIAAS) for fillets of flounder (*Platichthys flesus*, n=7), lemon sole (*Microstomus kitt*, n=5), megrim (*Lepidorhombus whiffiagonis*, n=5), and thornback ray (*Raja clavata*, n=5) based on recommendations for adults set by FAO et al. [2007]. Scores above 1.0 indicate contents higher than recommendations (green cells), scores below 1.0 indicate content lower than recommendations (red cells).

Amino acid	FAO et al. [2007] recommendations (mg/g protein)	**Digestible indispensable amino acid ratios** Flounder	Lemon sole	Megrim	Thornback ray
His	15	1.2	1.4	1.4	1.2
Ile	30	1.5	1.5	1.6	1.6
Leu	59	1.4	1.4	1.4	1.5
Lys	45	2.2	2.3	2.3	2.2
Met + cys*	22	1.5	0.7	1.2	1.5
Phe + tyr	38	1.9	2.2	1.8	1.8
Thr	23	2.1	2.3	2.3	2.3
Trp*	6	-	-	-	-
Val	39	1.2	1.1	1.2	1.2
Total IAA	277	1.7	1.7	1.7	1.7
DIAAS		**120%**	**70%**	**120%**	**120%**

*Not measured in analysis.

In the calculation of DIAAS, the total TAA content in the species was used to convert from mg/g ww to mg/g protein. This means that the levels of non-measured amino acids in the analysis were not considered in the calculation. The DIAAS scores would have been lower if these amino acids were considered. Additionally, tryptophan was not measured in the analysis and can potentially be the limiting IAA in the species.

The contribution to all daily IAA for an 80 kg adult in a 200 g portion of fish was estimated, and the results are shown in fig. 4.1. The estimation was based on the amino acid with the lowest digestible IAA ratio in the different species (valine for the flounder, the megrim, and the thornback ray, and methionine for the lemon sole), and the Norwegian Health Directorate recommendation of a dinner serving of 200 g for lean fish [Dalane et al., 2015]. For the flounder and the megrim, a dinner serving of the fish provided an estimated 61% and 68% of the daily amino acid requirements for an 80 kg adult. The megrim, which had lower amino acid scores than the flounder, required a smaller portion due to higher total protein levels. For the thornback ray, a 200 g portion provided around half of all IAA needed for the day, while the lemon sole provided around one-third of total requirements. Although the total contribution of the lemon sole and the thornback ray was lower than for the flounder and the megrim, a dinner serving of these species still provided a significant contribution to TAA requirements.

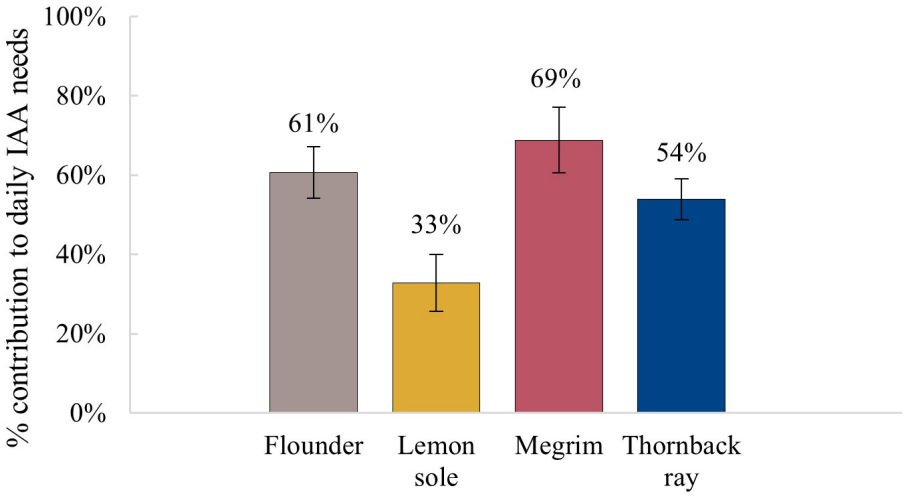

Figure 4.1: The contribution of all indispensable amino acids to daily requirements for an 80 kg adult in a 200 g portion of fillet. The contribution is estimated for flounder (*Platichthys flesus*, n=7), lemon sole (*Microstomus kitt*, n=5), megrim (*Lepidorhombus whiffiagonis*, n=5), and thornback ray (*Raja clavata*, n=5), and based on recommendations from FAO et al. [2007].

4.3 Free amino acids

FAA analysis was conducted on fillets of flounder (*P. flesus*), lemon sole (*M. kitt*), megrim (*L. whiffiagonis*), and thornback ray (*R. clavata*). The FAA results are graphically shown in fig. 4.2 and classified according to the basic taste of the FAAs following Fuke and Konosu [1991], Kirimura et al. [1969], Sarower et al. [2012]. For more detailed data, see appendix D.

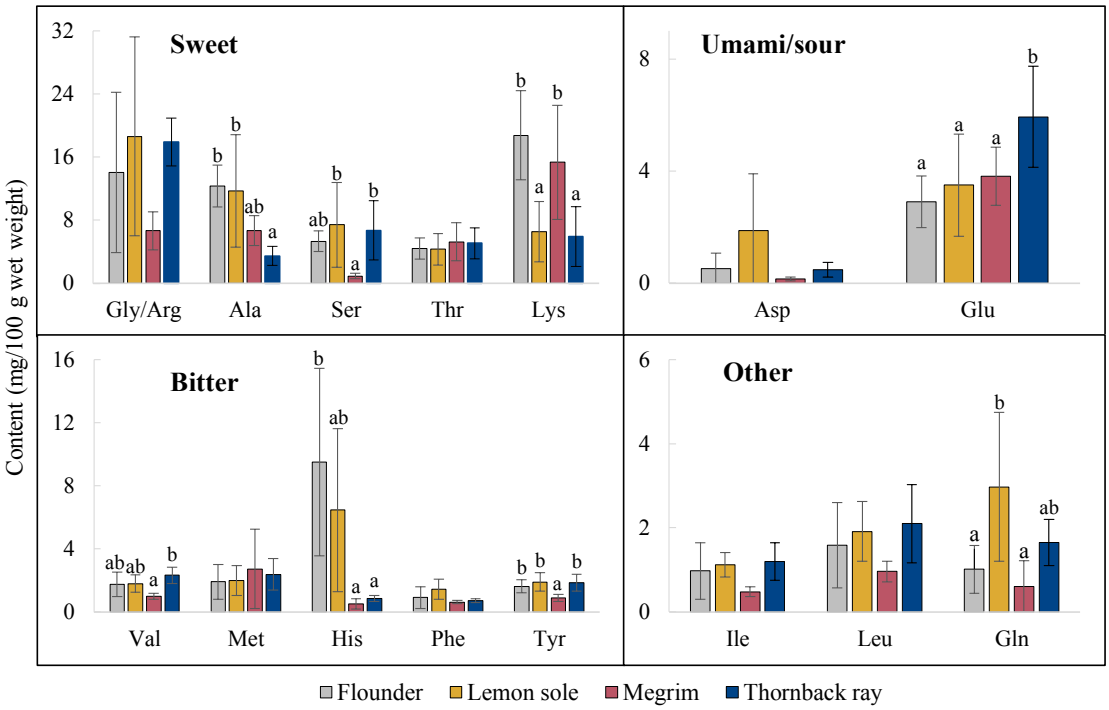

Figure 4.2: Free amino acid content for central fillets of flounder (*Platichthys flesus*, n=7), lemon sole (*Microstomus kitt*, n=5), megrim (*Lepidorhombus whiffiagonis*, n=5), and thornback ray (*Raja clavata*, n=5). Classification of free amino acids according to their basic taste following Fuke and Konosu [1991], Kirimura et al. [1969], Sarower et al. [2012]. Superscript letters represent a significant difference between species (α=0.05). Asparagine is not shown due to shallow contents.

Glycine/arginine and alanine were among the most prevalent FAAs for all the flatfish species. Lysine was the most abundant FAA for the flounder and the megrim, while serine was abundant in the lemon sole and the thornback ray. Next to these FAA, taurine was found to be the most abundant FAA in previous studies [Jones, 1959, Karl et al., 2013]. Taurine constituted about half of the FAA content found by Karl et al. [2013] but was not investigated in this study.

Among umami/sour amino acids, the thornback ray contained significantly higher glutamic acid levels than all other species ($P = 0.01$). Among sweet amino acids, the flounder and the megrim contained higher levels of lysine than the two others ($P = 0.001$). The differing lysine content between species was correlated with findings from Karl et al. [2013], which found higher levels of lysine in Alaskan soles than plaice. The lemon sole

contained higher levels of alanine than the thornback ray ($P = 0.003$) and higher levels of serine than the megrim ($P = 0.02$). Statistical differences were found for three of five bitter amino acids. The megrim contained lower levels of tyrosine compared to all other species ($P = 0.01$) and valine compared to the thornback ray ($P = 0.01$). The flounder had higher levels of histidine than the megrim and the thornback ray ($P = 0.004$), around 10 times as much on average, while the lemon sole had intermediate levels. A differing histidine content between species was also found by Karl et al. [2013] where plaice contained high levels of the FAA. For other amino acids, the lemon sole contained significantly higher glutamine levels than the flounder and the megrim ($P = 0.006$).

In total, the average FAA content of the megrim and the thornback ray was lower than for the flounder and the lemon sole, although not statistically significant. The total FAA contents found by Karl et al. [2013] for Alaskan soles and plaice were two to four times higher than the FAA contents found in this study when excluding taurine from the total. The present study's lower levels of FAA can be linked to freshness, as FAA content increases during storage due to protein breakdown. The fish used in this study was caught in a research vessel and frozen immediately after capture and filleting, while the fish from the investigation by Karl et al. [2013] was procured through markets and suppliers. For the lemon sole, the FAA contents were in the same range as those found by Jones [1959], although generally lower. The total FAA content did not reflect the total protein content as it did for TAA. Notably, the megrim, which contained the highest TAA levels, contained, on average, the lowest FAA levels (not significant).

Overall, the species contained five to seven times higher sweet amino acid concentrations than umami/sour and bitter amino acids. For the thornback ray and the megrim, the concentration of bitter and umami/sour amino acids was roughly the same, while the lemon sole and the flounder had about three times the concentration of bitter FAAs compared to umami/sour ones. As such, the lemon sole and the flounder had significantly higher bitter amino acid levels than the megrim and the thornback ray ($P < 0.001$). However, the results cannot be used to predict specific tastes, as the importance and contribution of individual amino acids to the taste differs with species [Sarower et al., 2012]. Additionally, amino acids have synergistic and antagonistic effects, and individual amino acids can be described by more than one flavor profile [Fuke and Konosu, 1991]. For example, bitter amino acids are not necessarily undesirable, as several can enhance umami flavor and provide the characteristic taste of the product [Sarower et al., 2012]. To obtain more apparent links between FAA content and the taste of fresh fish, a sensory evaluation must be conducted to evaluate the flavor contribution of a single FAA.

The FAA contents had high variance within the species, with a fractional uncertainty for total FAA content ranging from 13.0% for the thornback ray to 43.0% for the lemon sole. The variance was generally lower for the thornback ray and higher for the lemon sole and the megrim. Within samples, parallels had a low standard deviation, and external factors may explain the high variance. This includes factors such as sex, maturity, and feeding behavior [Jones, 1959]. For the lemon sole and the flounder, part of the variance could also be explained by season. Some representatives had been caught in September,

while others in April for these species. The lemon sole samples caught in April (n=3, 92.1 mg/100 g ww) had twice the amount of total FAA compared to the samples caught in September (n=2, 46.0 mg/100 g ww). For the flounder, the differences were more minor, where the April samples contained a total FAA average of 89.4 mg/100 g ww (n=2) and the September samples 73.1 mg/100 g ww (n=5). The differences between seasons were not investigated statistically due to insufficient sample sizes. However, the same seasonal variation was found by Jones [1959] when investigating lemon sole in England, where peak FAA levels were found in February and March. Higher levels of glycine and glutamate were especially reported by Jones [1959], and the same tendency was observed in this study. Both male and female fish were used in this study, so the different sexes can also explain part of the variance observed for FAA. However, it was not noted which sample corresponded to which sex, so potential differences could not be investigated.

4.4 Fatty acids

The relative distribution of FAs in four fish species was investigated to examine the nutritional profile of the species. The results are shown in table 4.4. All species' most abundant FAs were C22:6 (DHA), C16:0, and C18:1 n9, in addition to C20:5 (EPA) for the flatfish representatives. The same FAs were found to be most abundant in previous investigations on flatfish and thornback ray, as seen in table 2.3.

Table 4.4: Fatty acid distribution for central fillets of flounder (*Platichthys flesus*, n=7), lemon sole (*Microstomus kitt*, n=5), megrim (*Lepidorhombus whiffiagonis*, n=5), and thornback ray (*Raja clavata*, n=5), shown with standard deviation.

Fatty acids[1]	Flounder	Lemon sole	Megrim	Thornback ray	p-value
C14:0	1.5 ± 0.0^{ab}	1.4 ± 0.3^{b}	2.6 ± 0.6^{c}	0.3 ± 0.3^{a}	< 0.001
C15:0	0.5 ± 0.1^{b}	0.4 ± 0.3^{ab}	0.3 ± 0.2^{ab}	0.1 ± 0.1^{a}	0.046
C16:0	20.3 ± 1.6	23.9 ± 6.2	20.1 ± 3.2	26.0 ± 1.6	-
C17:0	0.6 ± 0.1	1.1 ± 1.0	0.3 ± 0.2	0.4 ± 0.4	-
C18:0	5.4 ± 0.7	6.4 ± 1.1	4.6 ± 1.4	5.1 ± 0.3	-
Total SFA[2]	28.3 ± 2.2	33.2 ± 6.4	27.9 ± 3.5	31.8 ± 1.5	-
C14:1	0.0 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.0 ± 0.0	-
C16:1	2.8 ± 0.3^{a}	1.8 ± 1.6^{a}	4.7 ± 1.2^{b}	1.7 ± 0.1^{a}	0.003
C17:1	0.1 ± 0.2	0.4 ± 0.4	0.3 ± 0.2	0.0 ± 0.0	-
C18:1 n7	2.3 ± 0.3	1.8 ± 1.6	2.6 ± 0.7	3.5 ± 0.6	-
C18:1 n9	9.8 ± 2.4	12.2 ± 4.9	13.5 ± 2.7	8.4 ± 0.7	-
C20:1 (n9+n11)	1.9 ± 0.3^{a}	0.8 ± 0.9^{a}	5.2 ± 1.7^{b}	1.3 ± 0.7^{a}	< 0.001
C22:1	0.4 ± 0.6^{a}	0.1 ± 0.2^{a}	2.5 ± 2.0^{b}	0.0 ± 0.0^{a}	0.02
Total MUFA[2]	17.3 ± 2.5^{a}	17.2 ± 0.6^{a}	29.0 ± 4.3^{b}	14.9 ± 2.0^{a}	< 0.001
C16:2	0.7 ± 0.2	0.7 ± 0.6	0.5 ± 0.4	0.0 ± 0.0	-
C18:2 n6	2.9 ± 1.8	1.2 ± 1.0	0.9 ± 0.6	1.5 ± 0.2	-
C18:3	0.8 ± 0.3	0.4 ± 0.4	0.2 ± 0.4	0.1 ± 0.2	-
C18:4 n3	0.4 ± 0.3	0.2 ± 0.3	0.7 ± 0.5	0.0 ± 0.0	-
C20:2 n6	0.1 ± 0.2	0.4 ± 0.3	0.2 ± 0.1	0.0 ± 0.0	-
C20:4 n3	0.2 ± 0.3	0.2 ± 0.3	0.6 ± 0.4	0.1 ± 0.3	-
C20:6 n6	6.3 ± 1.6^{ab}	8.9 ± 2.5^{b}	2.9 ± 1.8^{a}	4.3 ± 0.6^{a}	0.003
C20:5 n3 (EPA)[3]	12.9 ± 2.4^{b}	14.9 ± 3.0^{b}	6.6 ± 2.3^{a}	4.0 ± 0.7^{a}	< 0.001
C22:5 n3 (DPA)[4]	2.0 ± 0.3	2.5 ± 2.2	2.3 ± 0.3	3.1 ± 0.8	-
C22:6 n3 (DHA)[5]	23.1 ± 3.9^{b}	14.7 ± 2.7^{a}	24.5 ± 2.4^{b}	36.2 ± 1.4^{c}	< 0.001
Total PUFA[2]	49.4 ± 2.4^{b}	44.6 ± 2.4^{ab}	39.5 ± 4.3^{a}	49.3 ± 2.0^{b}	0.002
Total n3	39.3 ± 4.4^{ab}	32.0 ± 2.6^{a}	35.2 ± 4.1^{a}	43.5 ± 1.8^{b}	0.002
n3/n6	4.5 ± 1.8^{a}	3.2 ± 0.6^{a}	9.3 ± 2.4^{b}	7.6 ± 0.9^{b}	0.001
Unidentified FAs[2]	4.0 ± 1.9	5.5 ± 5.3	2.9 ± 1.3	4.1 ± 1.4	-
Total	100	100	100	100	-

[1]Superscript letters represent significance (α = 0.05).

[2]Saturated- (S), monounsaturated- (MU), polyunsaturated- (PU) fatty acids (FAs).

[3]Eicosapentaenoic acid (EPA)

[4]Docosapentaenoic acid (DPA)

[5]Docosahexaenoic acid (DHA)

The species had a similar total SFA distribution, ranging from 28% to 33% on average. Statistical differences were found for two SFAs. The megrim had a significantly higher proportion of C14:0 than the other species ($P < 0.001$), while the flounder had a higher proportion of C15:0 than the thornback ray ($P = 0.05$). For the flounder and the lemon sole, the relative SFA levels were 2 pp to 11 pp higher than previous findings on similar species, while previous investigations of megrim found overlapping SFA proportions compared to the results from this study [Afonso et al., 2013]. The SFA results on the thornback ray of 30% to 33% were lower than in previous investigations, where up to 48% SFA was identified [Turan et al., 2007]. Low water temperatures could explain the lower relative SFA content of the thornback ray in this study. The previous investigations were conducted on skates caught in the Black Sea and North Aegan Sea near Turkey, where sea temperatures are higher than on the Norwegian coast (13.7 °C and 4 °C at capture). Previous studies have shown that same-species fish held at cold temperatures have enhanced levels of MUFA and PUFA compared to fish held at warmer temperatures [Fadhlaoui and Couture, 2016, Tocher et al., 2004]. Long and unsaturated FAs have low melting points that ensure fluid cell membranes in the fish, even in cold waters [Fadhlaoui and Couture, 2016]. The lower SFA levels of the thornback ray investigated in this study could thus be a result of this cell mechanism.

The megrim had a significantly higher MUFA proportion than the other investigated species, with an average proportion of 29% compared to 15% to 17% for the others ($P < 0.001$). The higher relative content was caused by generally higher levels of all identified MUFAs, with significantly higher proportions of three of seven MUFAs than all other species. The relative MUFA levels determined for the megrim and the thornback ray were similar to previous investigations of 22% to 31% for megrim by Afonso et al. [2013] and from 13% to 18% for thornback ray by Colakoglu et al. [2011] and Turan et al. [2007].

All species had fatty acid profiles dominated by PUFAs, making up from 34% to 53% of total FAs. The thornback ray and the flounder had a significantly higher PUFA proportion than the megrim ($P = 0.002$). The PUFA proportion was in correspondence with previous investigations on similar species, although a few pp higher in previous studies for relatives of flounder and lemon sole (46% to 52% for plaice and Alaskan soles) and a few pp lower in previous studies for the thornback ray (24% to 43%) and the megrim (23% to 30%) (table 2.3). The distribution of SFA, MUFA and PUFA in the total lipid content for the different species is shown in fig. 4.3.

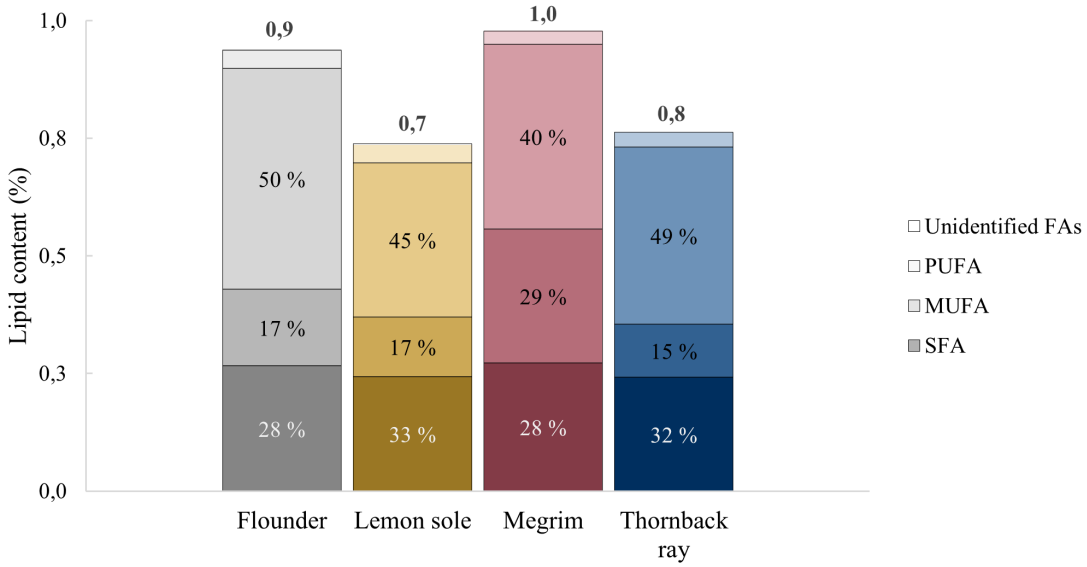

Figure 4.3: Relative average saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) proportions in central fillets of flounder (*Platichthys flesus*, n=7), lemon sole (*Microstomus kitt*, n=5), megrim (*Lepidorhombus whiffiagonis*, n=5),) and thornback ray (*Raja clavata*, n=5), shown with the total lipid content for the species.

There were interspecific variances within the distribution of different PUFAs. The thornback ray had the highest DHA proportion with an average proportion of 36%, while the megrim and the flounder had higher proportions than the lemon sole (from 23% to 25% against 15%) ($P < 0.001$). A reversed situation was observed regarding the relative EPA content. The flounder and the lemon sole contained the highest proportion with averages from 13% to 15%, significantly higher than the megrim and the thornback ray at 4% to 7% ($P < 0.001$). The lemon sole was the only species with similar EPA and DHA proportions, with the other species having twice as much or more DHA than EPA. The lemon sole also differentiated itself from the others with significantly higher relative levels of the n6 isomer of C20:4, compared to the megrim and the thornback ray ($P = 0.003$).

The findings indicate a few pp higher DHA and EPA levels for the megrim than previous investigations, which found 4% EPA and 15.5% DHA, with a comparable ratio of DHA:EPA [Afonso et al., 2013, Barbosa et al., 2018]. For the the flounder and the lemon sole, the findings were comparable with those of other flatfish species, as shown in table 2.3. The lemon sole was more similar in DHA and EPA distribution to plaice, while the flounder was more like yellow-tail flounder. The relative EPA levels of the thornback ray were similar to previous findings, while the relative DHA levels were two to three times as high in this study compared to previous ones [Colakoglu et al., 2011, Turan et al., 2007]. The high levels of DHA in this study could be linked to the cold-water mechanism described above.

Given the health aspects of n3, the total n3-content and n3/n6 ratio were investigated. The thornback ray had the highest total n3 average proportion with 43.5% of total FAs, signif-

icantly higher than the megrim and the lemon sole ($P = 0.002$). The flatfish representatives all had average n3 proportions in the 32% to 44% range, and all species contained a high proportion of the n3 FAs DHA and EPA. DPA was also present in all species. The high n3 findings indicate a beneficial FA distribution. All these three n3 FAs have reputed health effects, and previous studies have shown both shared and complementary effects of DHA and EPA, and growingly also for DPA. Increased consumption of these FAs is recommended as the current world intake is too low [Coultate, 2016, Helsedirektoratet, 2011, Mozaffarian and Wu, 2012, p. 121].

There were also some interspecific differences in the n3/n6 ratio, where the megrim and the thornback ray had a significantly higher ratio than the flounder and the lemon sole ($P = 0.001$). The megrim had the highest n3/n6 ratio, with an average of 9.3, even though it had the lowest overall PUFA content. The beneficial ratio of the megrim was thus caused by low relative levels of n6 rather than high relative levels of n3. The thornback ray had an average n3/n6 ratio of 7.6. In contrast, the flounder and the lemon sole had averages of 4.5 and 3.2, respectively. The findings corresponded with previous investigations on the megrim where ratios from 6 to 11 was found [Afonso et al., 2013]. For the lemon sole and the flounder, the findings were in the same range as previous studies on plaice and four-spotted megrim of five to four, respectively [Karl et al., 2013]. However, the found n3/n6 ratio of the thornback ray was much higher than in previous investigations, where averages of around four were determined [Colakoglu et al., 2011, Turan et al., 2007]. The calculated n3/n6 ratio indicated beneficial ratios for all fish. For the flounder and the lemon sole, the ratios were on the low end of ratios calculated for various flatfish species, while the megrim and the thornback ray displayed ratios on the high end (Table 2.3). The consumption of all the species would contribute towards the recommended n3/n6 rational intake of 1:6.

The total EPA+DHA intake during consumption of the species was calculated to investigate how the species provide these indispensable FAs in the human diet. The relative n3 distribution from the FAME analysis was converted to absolute values (g FA/100 g ww fillet) using conversion factors proposed by Weihrauch et al. [1977]. The contribution to daily recommendations of 500 mg EPA+DHA by EFSA [2012] in a dinner serving of the different species is shown in fig. 4.4, using a 200 g portion size as Dalane et al. [2015] recommended.

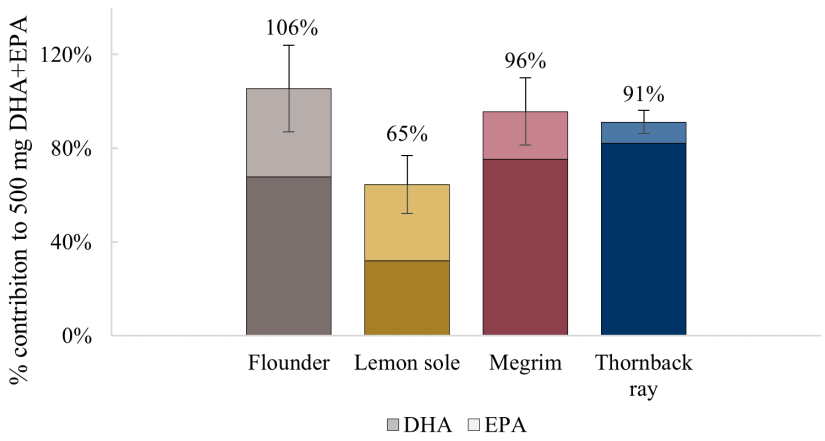

Figure 4.4: The contribution of a 200 g fillet of fish to the daily recommended intake of 500 mg DHA+EPA by EFSA [2012]. Shown for species flounder (*Platichthys flesus*, n=7), lemon sole (*Microstomus kitt*, n=5), megrim (*Lepidorhombus whiffiagonis*, n=5), and thornback ray (*Raja clavata*, n=5). The relative contribution of DHA and EPA in the fillets is shown in dark/light shading. The relative fatty acid contribution was converted to mg/100 g edible portion using conversion factors proposed by Weihrauch et al. [1977].

This estimation shows that one 200 g dinner serving of the flounder, the megrim, and the thornback ray covered on average from 91% to 106% of the daily recommended intake of DHA+EPA. The findings for the megrim was in the same range as the estimation done by Afonso et al. [2013], which found that, on average, a 190 g fillet would cover the daily EPA+DHA recommendations. These results demonstrate the significant contribution lean species can make to the n3 FA intake in the human diet. For the lemon sole, which was both on the low end on the relative n3 FA content and total lipid content, the fillet size needed was above a typical serving size. However, the total intake of EPA in a portion of the lemon sole is more significant than for the megrim and the thornback ray, and this fish could also contribute to n3 FA intake.

Even though the above-stated results promote these species as adequate n3 suppliers, the portion needed to cover daily DHA+EPA recommendations is estimated. The FAME analysis was conducted in singlicate, so there is a higher degree of uncertainty related to the results of this analysis compared to the other analyses conducted in the present thesis. The conversion factors used are also general for fish, which decreases estimation accuracy. Nevertheless, the results picture the potential of lean fish providing sufficient levels of n3 in a 200 g portion as recommended by EFSA [2012].

4.5 Final considerations on the chemical composition

The flounder, the lemon sole, the megrim, and the thornback ray had a broadly comparable chemical composition, with the same patterns in their proximate composition, TAA, FAA, and FAs. The lemon sole and the flounder had the most similar chemical composition in all analyses. This was expected given the close phylogenetic relationship and ecological niches of the fish. Furthermore, their proximate composition corresponded closely with investigations on other flatfish species in the same family, including plaice, yellowfin sole, and Alaskan soles.

The megrim, which does not belong to the same family as the other flatfish, was expected to be more dissimilar from the others. Indeed, the megrim differentiated itself from the others in TAA, FAA, and FA contents with a higher protein and TAA content and a different FA distribution. Surprisingly, the thornback ray, which is very distantly related to the three other fish species, was also characterized by the same broad features as the flatfish. It was equally dissimilar to the flounder and the lemon sole as the megrim, which points towards other factors besides phylogeny to dictate the chemical composition. The thornback ray has a similar ecological niche as the flatfish, feeding on the same diet and living in the same water temperatures. This may explain the comparable chemical composition.

Overall, the investigated fish species are highly nutritious. They contain a high protein content (more than 16%), which again contain a high level of IAAs. The megrim, the flounder, and the thornback ray were found to be protein sources of "excellent" quality meaning their amino acid distribution meet all nutritional requirements set for adults. The lemon sole was determined to be of poorer protein quality, as its protein profile was in deficiency of methionine but was still high in all other IAAs. Even though all the fish are lean to low-fat species, their FA distribution was favorable. The fish contained large proportions of PUFAs, especially DHA and EPA. An estimated regular dinner serving of flounder and megrim can provide both the daily recommendations of DHA and EPA and contribute substantially to protein needs. Although the lemon sole and the thornback ray contain a less favorable nutritional profile, consumption of the fish provides reasonable amounts of IAAs and FAs. From this point of view, higher consumption of all investigated species can be recommended. The nutritional aspects of the species can be used in marketing to the Norwegian population when trying to establish these species in the Norwegian market. This applies especially to the flounder, which receives low market prices in Norway today, despite its remarkable nutritional value.

Furthermore, this high nutritional quality is the same in all four central fillets of the three flatfish species. No differences between upper/lower or loin/belly fillets were discovered during the analysis of the flatfish. All fillets thus have the same chemical distribution for proximate composition, FAA, and TAA. This is beneficial from a food perspective, as the fillets do not need to be treated differently in the food chain. Even though fillet differences were not investigated for FAs, previous results by Barbosa et al. [2018] and Kendler [2022, work in progress] point toward a homogenous distribution also in this aspect. All fillets of the flatfish thus contain the same beneficial nutritional profile.

Although different central fillets did not affect the chemical composition, other external factors did. Some chemical components displayed more variation than others, and FAA, lipid, and FA content were more variable than overall proximate composition and TAA. The more varied FAA and FA composition indicate that these components are more influenced by external factors, such as season, sex, maturity, and feeding pattern, than TAA. The lemon sole generally displayed higher variation than the other species in most analyses. This could be linked to seasonal variation, as the individuals of the lemon sole were caught in two different seasons, whereas individuals of other species were caught during the same month (exception for two out of seven flounders). The seasonal tendency seen for the lemon sole may therefore also be present in other species.

Chapter 5

Conclusion and further work

The chemical composition of four species of little utilized fish in Norway was determined to increase knowledge of the species and examine their consumption's nutritional aspects. The species had a similar chemical composition, having a high protein content (17% to 24%) and being lean to low-fat species (0.7% to 1.0%). The megrim distinguished itself with its higher protein content than other flatfish and its higher lipid content than the lemon sole and the thornback ray. For the thornback ray, there was significant deviance between the protein results from the Kjeldahl and TAA analyses, providing uncertainty to the high protein content (24%) found in the Kjeldahl analysis. The TAA content of the species was dominated by leucine and lysine, and all species contained high levels of IAAs. The flounder, the megrim, and the thornback ray were determined to be protein sources of excellent quality based on their DIAAS above 100%, meaning their IAA distribution meet all requirements set by FAO et al. (2007). Lemon sole obtained a DIAAS of 70% due to low methionine content. This means that while a 200 g portion of the three former species contributed from 54% to 68% of all daily IAA requirements for an 80 kg adult, the lemon sole only contributed 33%. The overall FAA content of the species was lower than in previous investigations and showed a significant variance between specimens. The FA profile of all the species was dominated by PUFA and determined to be beneficial. The most abundant PUFAs were n3 FAs DHA and EPA, with a beneficial n3/n6-ratio in all the species. A 200 g serving portion of the flounder, the megrim, and the thornback ray covered on average from 91% to 106% of the daily recommended intake of 500 mg DHA+EPA. From a nutritional point of view, the megrim and the flounder displayed the most beneficial nutritional profiles. However, a dinner serving of all the species contributes substantially to both protein and lipid requirements, and increased consumption of all species can be recommended. The chemical composition and beneficial nutritional profile were the same in all four central fillets of the flatfish species, as investigation of the different fillets yielded no statistically significant results.

The further focus should be on establishing a profitable industry from the capture and sale of the investigated species. The beneficial nutritional profiles can be used as key points when developing and establishing the species in the Norwegian market. This is especially valid for the flounder, which has excellent nutritional quality in terms of both protein

and lipid content despite a low market selling price. Further focus on other bottlenecks related to stock size and distribution channels will also be essential to develop the species commercially. Additionally, factors that affect the chemical composition of flatfish and thornback ray remain to be determined. The seasonal variation tendency seen for the lemon sole may be further investigated also for the other species. The microbial safety of the fish was not determined in this master's thesis and could be subject to further work. The nutritional quality of the fish may be partly counterbalanced by environmental contamination of heavy metals, dioxins, and PCBs, that are commonly found in seafood.

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Appendix A

Ileal digestibility conversion factors

Ileal digestibility conversion factors for specific indispensable amino acids are shown in table A.1, as proposed by FAO [2012]. They were used in the calculation of DIAAS of the fish species. Where specific digestibility factors were not available for the given amino acid, the general digestibility factor for the protein was used.

Table A.1: Specific ileal digestibility factors for fish as proposed by FAO [2012].

Amino acid	Ileal digestibility factor for fish (%)
His	85
Ile	93
Leu	91
Lys	72
Met	–
Phe	83
Tyr	–
Thr	95
Val	90
Protein	91

Appendix B

Fatty acid conversion factors

Fatty acid conversion factors (FACF) for finfish were calculated for each species as shown in eq. (B.1), as proposed by Weihrauch et al. [1977].

$$\text{FACF (g FA per g lipid)} = 0.933 \text{ (g FA per g lipid)} - \frac{0.143 \text{ (g FA per 100 g food)}}{\text{Total lipids}} \tag{B.1}$$

Where:

– Total lipids (g lipid/100 g food): from Bligh and Dyer method.

Appendix C

Results from the fillet investigation of flatfish

Lower loin and upper body fillets from flounder (*P. flesus*), lemon sole (*M. kitt*), and megrim (*L. whiffiagonis*) was investigated for proximate composition, TAA, and FAA to increase knowledge of the species. The results from the proximate composition is found in table C.1, the results from TAA in Table C.2, and the results from FAA in Table C.3.

Table C.1: Proximate composition for lower loin (LL) and upper body (UB) fillets of flounder (*Platichthys flesus*, n=7), lemon sole (*Microstomus kitt*, n=5), and megrim (*Lepidorhombus whiffiagonis*, n=5).

	Flounder		**Lemon sole**		**Megrim**	
Fillet	LL	UB	LL	UB	LL	UB
Water (%)	82.3 ± 1.4	81.9 ± 1.1	81.5 ± 1.0	81.4 ± 1.0	79.2 ± 1.7	79.2 ± 1.1
Ash (%)	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.0
Protein (%)	16.8 ± 1.0	17.0 ± 1.1	17.4 ± 0.5	17.6 ± 0.8	19.5 ± 1.2	19.7 ± 1.2
Lipid (%)	0.9 ± 0.2	1.0 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	1.1 ± 0.3	0.9 ± 0.1

Table C.2: Total amino acid contents for lower loin (LL) and upper body (UB) fillets of flounder (*Platichthys flesus*, n=7), lemon sole (*Microstomus kitt*, n=5), and megrim (*Lepidorhombus whiffiagonis*, n=5), shown with standard deviation.

	Flounder		**Lemon sole**		**Megrim**	
Fillet	LL	UB	LL	UB	LL	UB
His	2.7 ± 0.4	2.7 ± 0.5	3.2 ± 0.2	3.0 ± 0.8	3.2 ± 0.6	4.1 ± 1.7
Ile	5.8 ± 0.6	5.7 ± 0.8	6.1 ± 0.5	5.3 ± 1.1	6.7 ± 0.7	7.4 ± 0.8
Leu	11.3 ± 1.3	11.2 ± 1.6	11.7 ± 1.0	9.6 ± 2.6	12.9 ± 1.2	13.4 ± 0.8
Lys	13.6 ± 1.6	13.4 ± 2.1	14.4 ± 1.3	13.0 ± 3.1	16.1 ± 1.5	16.5 ± 0.9
Met	4.6 ± 0.6	4.5 ± 0.6	2.3 ± 0.5	1.6 ± 1.2	3.8 ± 1.1	4.7 ± 1.5
Phe	5.6 ± 0.6	5.6 ± 0.8	5.9 ± 0.4	6.3 ± 3.1	6.6 ± 0.7	6.9 ± 0.3
Tyr	5.2 ± 0.7	5.1 ± 0.8	5.2 ± 0.5	8.3 ± 8.4	5.8 ± 0.6	5.4 ± 1.7
Thr	6.7 ± 0.9	6.5 ± 1.1	7.2 ± 0.6	6.7 ± 1.9	8.1 ± 0.9	8.8 ± 0.6
Val	6.3 ± 0.7	6.3 ± 0.9	6.5 ± 0.5	5.0 ± 1.8	7.2 ± 0.8	7.1 ± 1.1
Sum IAA*	61.8 ± 7.3	61.1 ± 9.1	62.6 ± 5.5	58.7 ± 16.5	70.4 ± 7.0	74.4 ± 1.8
Asp	14.0 ± 1.6	13.9 ± 2.0	14.1 ± 1.1	13.0 ± 3.3	15.9 ± 1.6	16.3 ± 1.3
Glu	18.1 ± 2.1	17.8 ± 2.5	19.0 ± 1.8	17.6 ± 4.4	20.7 ± 1.9	21.4 ± 1.6
Asn	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.2 ± 0.4
Ser	7.5 ± 1.2	7.3 ± 1.4	8.9 ± 0.9	8.1 ± 2.6	8.6 ± 1.0	8.2 ± 2.4
Gln	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.5 ± 0.1	1.5 ± 2.2
Gly/Arg	8.1 ± 1.0	7.8 ± 1.1	8.7 ± 0.5	7.9 ± 2.3	9.0 ± 0.9	9.0 ± 1.1
Ala	8.9 ± 1.0	8.9 ± 1.3	8.7 ± 0.6	6.0 ± 3.7	10.1 ± 1.1	9.8 ± 2.4
Sum total	118.6 ± 13.8	117.0 ± 17.3	122.1 ± 10.5	111.5 ± 29.4	135.1 ± 13.5	140.8 ± 6.6

Table C.3: Free amino acid content for lower loin (LL) and upper belly (UB) fillets of flounder (*Platichthys flesus*, n=7), lemon sole (*Microstomus kitt*, n=5) and megrim (*Lepidorhombus whiffiagonis*, n=5), shown with standard deviation. Classification into groups of umami, sweet, and bitter-tasting amino acids based on Fuke and Konosu [1991], and Kirimura et al. [1969].

	Flounder		**Lemon sole**		**Megrim**	
Fillet	LL	UB	LL	UB	LL	UB
Asp	0.4 ± 0.4	0.7 ± 0.8	1.8 ± 2.2	1.8 ± 2.1	0.2 ± 0.1	0.1 ± 0.1
Glu	3.0 ± 1.0	2.8 ± 1.1	3.5 ± 2.3	2.8 ± 1.8	4.1 ± 1.2	3.5 ± 0.9
Asn	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sum sour/umami	3.4 ± 1.2	3.5 ± 1.1	5.4 ± 4.5	4.6 ± 3.4	4.3 ± 1.3	3.7 ± 1.0
Gly/Arg	13.7 ± 9.9	14.4 ± 10.5	17.3 ± 12.7	18.4 ± 14.1	6.7 ± 2.4	6.6 ± 2.4
Ala	12.3 ± 2.4	12.3 ± 3.0	12.2 ± 6.5	11.8 ± 7.4	7.2 ± 1.7	6.3 ± 2.1
Ser	5.3 ± 1.0	5.3 ± 1.8	8.1 ± 7.1	5.9 ± 4.5	1.0 ± 0.4	0.8 ± 0.2
Thr	4.1 ± 1.3	4.7 ± 1.5	4.2 ± 2.0	4.3 ± 2.2	5.4 ± 2.3	5.1 ± 2.6
Lys	21.0 ± 4.4	16.6 ± 8.1	7.2 ± 4.7	5.6 ± 3.4	15.2 ± 7.1	15.5 ± 7.4
Sum sweet	56.4 ± 10.9	53.4 ± 14.5	49.0 ± 28.6	45.9 ± 28.6	35.5 ± 11.2	34.3 ± 12.4
Val	1.8 ± 0.8	1.7 ± 0.8	1.7 ± 0.7	1.6 ± 0.6	1.0 ± 0.1	1.0 ± 0.2
Met	2.0 ± 1.1	1.8 ± 1.1	2.1 ± 0.9	2.0 ± 0.9	2.8 ± 2.4	2.7 ± 2.6
His	7.1 ± 4.2	11.9 ± 9.0	7.6 ± 5.5	6.3 ± 5.0	0.6 ± 0.4	0.4 ± 0.3
Phe	0.9 ± 0.7	0.9 ± 0.7	1.5 ± 0.7	1.4 ± 0.6	0.6 ± 0.1	0.6 ± 0.2
Tyr	1.6 ± 0.5	1.6 ± 0.3	1.9 ± 0.6	1.8 ± 0.7	0.9 ± 0.2	0.9 ± 0.3
Sum bitter	13.4 ± 3.1	17.9 ± 7.0	14.8 ± 3.4	13.1 ± 3.4	5.9 ± 2.9	5.6 ± 2.9
Ile	1.0 ± 0.7	0.9 ± 0.7	1.1 ± 0.4	1.0 ± 0.3	0.5 ± 0.1	0.5 ± 0.1
Leu	1.6 ± 1.0	1.5 ± 1.1	1.8 ± 1.0	1.6 ± 0.7	1.0 ± 0.2	0.9 ± 0.3
Gln	1.2 ± 0.6	0.8 ± 0.6	3.0 ± 1.8	3.0 ± 1.8	0.6 ± 0.6	0.6 ± 0.6
Sum others	3.9 ± 1.6	3.3 ± 1.8	5.9 ± 2.9	5.7 ± 2.4	2.1 ± 0.9	2.0 ± 1.0
Sum total	77.3 ± 12.9	78.2 ± 13.1	75.0 ± 34.0	69.4 ± 32.9	47.9 ± 14.2	45.6 ± 15.5

Appendix D

Results from the free amino acid analysis

Free amino acids were investigated for flounder (*P. flesus*), lemon sole (*M. kitt*), megrim (*L. whiffiagonis*) and thornback ray (*R. clavata*) to increase knowledge on the species. The results from the analysis is shown in table D.1.

Table D.1: Free amino acid content for central fillets of flounder (*Platichthys flesus*, n=7), lemon sole (*Microstomus kitt*, n=5), megrim (*Lepidorhombus whiffiagonis*, n=5), and thornback ray (*Raja clavata*, n=5), shown with standard deviation. Classification into groups of umami, sweet, and bitter-tasting amino acids based on Sarower et al. (2012), Fuke and Konosu (1991), and Kirimura et al. (1969).

Free amino acid content (mg FAA/100 g wet weight)*					
Amino acid	Flounder	Lemon sole	Megrim	Thornback ray	p-value
Asp	0.5 ± 0.6	1.9 ± 2.0	0.2 ± 0.1	0.5 ± 0.3	-
Glu	2.9 ± 0.9^a	3.5 ± 1.8^a	3.8 ± 1.0^a	5.9 ± 1.8^b	0.013
Asn	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	-
Sum sour/umami	3.4 ± 1.1	5.4 ± 3.6	4.0 ± 1.1	6.6 ± 1.9	-
Gly/Arg	14.1 ± 10.1	18.6 ± 12.6	6.7 ± 2.4	17.9 ± 3.0	-
Ala	12.3 ± 2.6^b	11.7 ± 7.1^b	6.7 ± 1.9^{ab}	3.5 ± 1.2^a	0.003
Ser	5.3 ± 1.3^{ab}	7.4 ± 5.4^b	0.9 ± 0.3^a	6.7 ± 3.8^b	0.021
Thr	4.4 ± 1.3	4.3 ± 2.0	5.3 ± 2.4	5.1 ± 1.9	-
Lys	18.8 ± 5.7^b	6.5 ± 3.8^a	15.3 ± 7.2^b	5.9 ± 3.8^a	0.001
Sum sweet	54.9 ± 12.1	48.6 ± 27.2	34.9 ± 11.7	39.1 ± 12.1	-
Val	1.7 ± 0.8^{ab}	1.8 ± 0.5^{ab}	1.0 ± 0.2^a	2.3 ± 0.5^b	0.014
Met	1.9 ± 1.1	2.0 ± 0.9	2.7 ± 2.5	2.4 ± 1.0	-
His	9.5 ± 6.0^b	6.5 ± 5.2^{ab}	0.5 ± 0.3^a	0.8 ± 0.2^a	0.004
Phe	0.9 ± 0.7	1.4 ± 0.6	0.6 ± 0.1	0.7 ± 0.2	-
Tyr	1.6 ± 0.4^b	1.9 ± 0.6^b	0.9 ± 0.2^a	1.8 ± 0.5^b	0.01
Sum bitter	15.7 ± 4.1^b	13.6 ± 3.6^b	5.7 ± 2.9^a	8.1 ± 1.1^a	< 0.001
Ile	1.0 ± 0.7	1.1 ± 0.3	0.5 ± 0.1	1.2 ± 0.4	-
Leu	1.6 ± 1.0	1.9 ± 0.7	1.0 ± 0.2	2.1 ± 0.9	-
Gln	1.0 ± 0.6^a	3.0 ± 1.8^b	0.6 ± 0.6^a	1.7 ± 0.5^{ab}	0.006
Sum other	3.6 ± 1.7^{ab}	6.0 ± 2.5^b	2.0 ± 1.0^a	5.0 ± 1.9^{ab}	0.006
Sum total	77.8 ± 12.4	73.7 ± 31.7	46.8 ± 14.8	59.2 ± 9.0	

*Superscript letters represent significance between species (α=0.05).

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