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Fishery by-products, *Calanus finmarchicus* and mesopelagic fish species as alternatives to fish meal and fish oil in feeds for Atlantic salmon (*Salmo salar L.*).

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Abstract

Norway aims to become the world's leading seafood nation with a production of 5 million tons of aquaculture products by 2050. The demand for feed will increase accordingly. The world's fish resources are already fully exploited or overexploited and increased use of plant-based ingredients may not be considered sustainable. The aim of this thesis was therefore to investigate the potential for increased use of three marine derived feed ingredients. Fishery by-products, *C. finmarchicus* and mesopelagic fish are marine ingredients, that have potential to replace fish meal and fish oil in aquafeeds for Atlantic salmon. They have generally a favorable nutritional profile, and contain all the nutrients needed to satisfy the nutritional requirements of Atlantic salmon. Fish meal produced from by-products have similar nutritional profile to fish meal produced from reduction fisheries. *C. finmarchicus* have a high protein content and a well-balanced amino acid profile, favorable for salmonids. Mesopelagic fish species have a protein content slightly lower than fish meal with an essential amino acid composition close to that of fish meal. The lipid content of fisheries by-products, *C. finmarchicus* and mesopelagic fish species may vary among species and season. They are rich in the n-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA), especially EPA and DHA. There are also some limitations with these ingredients. Fish meal produced from by-products have high ash content. Mesopelagic fish species may contain several contaminants and heavy metals that may limit the incorporation level in feed. The lipids in *C. finmarchicus* and *B. glaciale* are stored as wax esters, that are poorly digested at high incorporation level. Available volumes of by-products from the Norwegian fisheries is relatively low, but full utilization of all by-products should still be a goal. The current harvesting technology and cost of processing zooplankton into oil and meal is limiting the potential of using zooplankton as feed ingredients. The nutritional composition of *C. finmarchicus* makes it a promising ingredient that can be important as a future feed ingredient. The enormous biomass of mesopelagic fish represent a big potential as a future feed ingredient in salmon feed. This resource is however, one of the least investigated in terms of distribution, abundance, fishing methods and product development. Sustainable management is deemed necessary before mesopelagic fish stocks can be harvested and used as a feed ingredient in feeds for salmon.

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1. Introduction

1.1 Development of aquaculture

Aquaculture production is increasing worldwide. It now accounts for more than half of the total food fish supply and the percentage is increasing every year (FAO, 2017). More than 220 species of fish and shellfish are being farmed (FAO, 2012). Global aquaculture production in 2016 was 110,2 million tons, it included 80,0 million tons of food fish and 30,1 million tons of aquatic plants as well as 37 900 tons of non-food products (ornaments). Farmed food fish production included 54,1 million tons of fin fish, 17,1 million tons of mollusks, 7,9 tons of crustaceans and 938 500 tons of other aquatic animals (turtles, sea cucumbers, sea urchins, sea frogs and edible jellyfish (FAO, 2017).

The Norwegian aquaculture production is small in the global scale but is characterized by production of highly valuable species. In 2018 the total production from Norwegian aquaculture reached 1 452 928 kg, of which 1,36 tons salmon *Salmo salar* were produced (SSB).

1.2 Fish meal and fish oil

Growth in aquaculture production is expected to increase in the future and more aquafeeds will be needed to feed the growth. More than 70% of the total global aquaculture production depends on supply of external feed inputs (Tacon, 2015). Diets for farmed fish have primarily been based on marine resources. Pelagic fish species such as anchovies, sardines, herring and mackerel have been traditionally used as ingredients for fish meal and fish oil. In 1994, 30 million metric tons (mmt) of reduction fish (1/3 of the global fish catch) have been removed from the marine food web to produce fish meal and fish oil for animal feeds and other industrial purposes (Naylor *et al.*, 2009). The use of fish oil and fish meal in aquaculture is more than tripled from 1992 to 2006 (Naylor *et al.*, 2009). In 1992 aquaculture consumed around 15% and 20% of global fish meal and fish oil supplies respectively (Tacon, 2008). In 2006 the numbers were 68% of available fish meal and almost 89% of available fish oil (Tacon and Metian, 2009).

Globally, 31.4% of fish stocks assessed are classified as overfished by the Food and Agriculture Organization of the United Nations (FAO, 2017). The proportion of stocks overfished varies geographically from 40% in the southeast Pacific, southwest Atlantic and across the eastern and

western central Atlantic and 59% in the Mediterranean and Black Sea (Blanchard *et al.*, 2017). Limited supplies and increasing awareness among consumers and governments of the ecological consequences and environmental impacts of human activities, have been forcing the necessity to mitigate pressure on reduction fisheries and find alternative ingredients of protein and oil from more sustainable sources (Naylor *et al.*, 2000, Deutch *et al.*, 2007, Tacon and Metian, 2008).

1.3 Plant- based protein and oil as alternatives to fish meal and fish oil

Plant- based protein and oil sources have been identified to have the greatest potential to replace fish meal protein and fish oil. Different plant derived meals and concentrates from oilseeds, such as soybean, rapeseed (canola) and sunflower; grains, wheat and corn glutens as well as legumes (beans, peas, and lupins) have all been explored as fishmeal and fish oil replacement with varying degrees of success (Forster *et al.*, 1999; Barrows *et al.*, 2007; Gaylord *et al.*, 2009). Soy protein is a global commodity and cost effective substitute for marine protein. Soy protein has a favorable amino acid profile and is commonly accepted, both qualitatively and quantitatively, by most fish species (Watanabe, 2002). In 1990 approximately 90% of the ingredients in Norwegian salmon feed were from marine origin. The use of marine ingredients in fish diets were reduced to around 30% in 2013, and was further reduced to 25% in 2016 (Ytrestøyl *et al.*, 2015, Aas *et al.*, 2019,).

A fish-in-fish-out ratio, i.e a weight-equivalent unit of wild fish used to produces a unit of cultured fish, is an important indicator for the use of marine ingredients to produce new fish products. For the aquaculture sector as a whole, the ratio of wild fish-in to farmed fish- out (FIFO) based on feed ingredients has been reduced from 1.04 in 1995 to 0.22 in 2015 (IFFO). For salmonid production, the FIFO ratio has decreased to 0.82. Marine nutrient dependency ratios which stands for the amount of marine oil and protein required to produce 1 kg of salmon oil and protein was 0.7 in 2013 (Ytrestøyl *et al.*, 2015). Thus, 0.7 kg of marine protein was used to produce 1 kg of salmon protein, showing that the Norwegian farmed salmon is a net producer of marine protein.

1.3.1 Nutritional limitations

Compared to marine ingredients, plants have certain nutritional limitation. Plant protein ingredients have relatively low crude protein, high crude fiber content, presence of anti-nutritional factors and plant oil is lacking the long chained polyunsaturated fatty acids (LcPUFA) (Ruyter *et al.*, 2000, Hemre *et al.*, 2007, Kaushik, 2008, Krogdahl *et al.*, 2010, NRC, 2011; Bou *et al.*, 2017, Cheng *et al.*, 2018).

The amino acid profile in plant protein differ from fishmeal. Plant proteins are often low in lysine and methionine compared to fish meal (Gatlin *et al.*, 2007). Several studies have investigated the potential of plant protein ingredients to replace fish meal. Some studies have concluded that marine ingredients can be replaced without negative effects on growth performance, nutrient utilization and health of fish (Kaushik, 1990, Rodehutsord *et al.*, 1995, Refstie S *et al.*, 2000, Espe *et al.*, 2006, 2007, Burr *et al.*, 2012, Metochis *et al.*, 2017). Other studies however, have shown negative effects (Kaushik *et al.*, 2004, Barrows *et al.*, 2007, Urán *et al.*, 2009, Burr *et al.*, 2012).

Plant oils are typically rich in C18:2- 6 poly unsaturated fatty acids and monounsaturated fatty acids (MUFA), but lack the n-3 long chain polyunsaturated fatty acids EPA and DHA that are characteristic of marine fish oil (Turchini *et al.*, 2011). Marine fish species have limited capacity to synthesize eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (ARA; 20:4n-6) from α -linolenic acid (ALA; 18:3n-3) and linoleic acid (LA; 18:2n-6), therefor the long chain polyunsaturated fatty acids have to be added to the feeds (Glencross, 2009, Tocher, 2010). In addition to lower content of n-3 long chain polyunsaturated fatty acids, a higher inclusion of plants oil results in unfavourable n-3/n-6 ratio in aqua feeds, associated with compromised fish health (Urán, 2009, Praatoomyot *et al.*, 2011, Bou *et al.*, 2017, Sissener *et al.*, 2018).

Plant ingredients have high carbohydrate content and a wide range of antinutritional factors, including protease inhibitors, lectins, saponins and phytic acid. (Krogdahl *et al.*, 2010) The ability of fish to utilize dietary carbohydrates as energy sources varies, both among and within species. Carnivorous fishes have limited capacity to utilize complex carbohydrates and high amount of starch in the feed, compared to herbivorous fishes. The main reason for this is that carnivore fish have a digestive apparatus adapted to utilize protein and lipid in the feed. High level of digestible starch in the diet reduce digestibility and result in reduced growth of salmonids (Xie *et al.*, 1973, Hemre *et al.*, 1995).

Phytic acid, which is the major phosphorus component in plant-based ingredients affects the digestibility of protein and availability of phosphorous and other dietary elements (Spinelli *et al.*, 1983, Storebakken *et. al.*, 1998, Sajjadi *et al.*, 2004). Digestibility of phytate phosphorus is very poor, since fish do not possess phytase in the digestive tract. (Hua, 2006, Denstadli *et al.*, 2006, Kumar *et al.*, 2012). Phytic acid can bind to Zn²⁺, Fe²⁺ or Ca²⁺ ions, and by forming salts with these metals, decrease their intestinal absorption and utilization (Hua, 2006). Replacing dietary fish meal by plant feedstuffs rich in low digestible phytate, most of the phytate-P ends up excreted into the water, resulting in water eutrophication. Most of the antinutritional factors are easily removed from plant ingredients during production, phytic acid, however, require enzymatic treatment with phytase.

Another challenge when fish meal and fish oil are substituted by plant ingredients, is the influence on nutrient content or flesh quality. A number of studies have shown that replacing fish oil, which is the main dietary source of the n-3 long-chain polyunsaturated fatty acids, with plant oils, has influenced nutritional value of the final product (Rosenlund *et al*, 2001, Rørå *et al.*, 2005, Menoya *et al.*, 2010, Sissener *et al*, 2018). Gatlin (2007) showed that 40% of the studies that investigated effects of plant protein sources on flesh quality (flavor, color, odor and texture), reported significant effect.

1.3.2 Environmental and socio-economic effects

Use of plant ingredients reduce the pressure on wild fish stocks and used to be promoted as a sustainable alternative to the marine ingredients (Tacon and Foster, 2003, Tacon and Metian, 2008, 2009). However, the production of terrestrial feed ingredients can be associated with land use intensification, high energy-dependency ratios, greenhouse gas emissions, and significant pressure on freshwater resources (Boissy *et al.*, 2011, Troell *et al.*, 2014, Pahlow *et. al.*, 2015, Blanchard *et al.*, 2017, Malcorps *et al.*, 2019). Many of these crops are also consumed directly by humans and provide essential nutrition for low-income household. High pressure on these resources for aquafeeds use can potentially increase price levels and volatility, aggravating food insecurity among the most vulnerable populations (Troell *et al.*, 2014). Increased pressure on crop resources may have adverse socio-economic and environmental effects, and is arguing against increased use of plant-based ingredients in aquafeeds.

1.4 Low trophic and microbial ingredients

The negative environmental footprint associated with use of plant ingredients has intensified the research for novel ingredients that are not directly consumed by humans and which can be produced without use of agricultural land. Novel ingredients that may be used as substitutes for fish meal and fish oil in diets for carnivorous fish such as Atlantic salmon are microalga, such as diatoms and dinoflagellates (Tibbetts *et al*, 2011, Kiron *et al*, 2016, Sørensen *et al*, 2016), insect meal and oil (Belghit *et al*, 2018, Bruni *et al*, 2019), euphausiids (Olsen *et al*, 2006, Olsen *et al.*, 2011, Kousoulaki *et al*, 2013) and calanoid copepods (Olsen *et al*, 2004, Olsen, 2010). Single-cell proteins have also been used as alternative resources to a different extent. (Gatlin *et al.*, 2007, Naylor *et al.*, 2009, Caballero-Solares *et al.*, 2017).

1.5 Aim of this thesis.

More than 90% of ingredients used in Norwegian aquafeeds are imported (Sjømat Norge). Many factors can directly or indirectly affect the availability of these resources, starting from political conflicts, pandemics and natural disasters, but also smaller local challenges, can jeopardize availability and market situation. Future growth of Norwegian salmon production should take place with more use of ingredients produced in Norway. Exploring potential of novel feed ingredient includes assessment of :

- availability, both in volume, but also regulatory,
- nutritional quality
- possibility to handle and use in feed production.

In this thesis I will focus on the resources that are available in large quantities and not currently utilized to a large extent in Norwegian aquafeed: by-products from commercial gadiform fisheries, *Calanus finmarchicus* and mesopelagic fish species. All the three resources can be obtained in the Norwegian waters and by Norwegian shareholders. By-products are already commercially used in aquafeeds but still there is a potential to increase the utilization. *Calanus finmarchicus* is utilized in production of niche commodities by one commercial stakeholder. Knowledge about mesopelagic fish species is still scarce, but is expected to have a great potential for commercial use. The aim of this thesis is to investigate the potential of the novel marine ingredients as future ingredients in feeds for salmonids.

2. Fishery by-products

The Norwegian government has an ambition that the seafood industry should aim for a higher degree of utilization of by-products, and the goal is to bring to land and utilize 100% of the by-products (Norwegian white paper 10 (2015-2016), Norwegian white paper 45 (2016-2017), Blue opportunities, 2019). It is however not implemented by law, as it should be technically achievable and economically profitable. The high demand for marine ingredients should be a high motivation for the industry to use more by-products, however, still there is too much unexploited by-products in Norway and world - wide.

2.1 By-product: definition

The EC regulation on animal by-products (EC Nr 1069/2009 adopted on 21 October 2009), and Norwegian regulation for animalia by-product (animaliebiproduktforskriften), adopted on 14. September 2016, define animal by-products as whole carcasses or parts of animals or products not intended for human consumption. Today the definition is used on all by-products, edible or not intended for human consumption, left during the production of the main product (Rustad, 2007). The term includes the side streams from slaughtering of the fish and left over after filleting. The main by-products in the fisheries sector includes heads, liver, gonads, trimmings, backbone, skin, bones, stomachs and swimming bladder. 'Waste' are defined as products that cannot be used for feed and must go for destruction.

A general definition of by-products includes also discards or by-catches. 'Discards' or by-catch refer to fish that is caught and subsequently thrown overboard and includes undersized marketable species, commercial species that are unwanted due to legislation, low market value or any other reason, and non-commercial species ranging from benthic invertebrates to cephalopods and fish (Bellido *et al.*, 2011). Discards of most fish and crustacean species have been prohibited in Norway since 1987, while the EU has implemented mandatory landing for all species of fish in 2019. (Regulation (EU) No 1380/2013). Also in the regulatory comprehension, there is a difference between by-products that can be used for further consumption, discards and waste (Rustad *et al.*, 2003)

In this thesis by-products refer to all types of by-products, that is left after processing of fish for human consumption, and can be recycled after treatment.

2.2 Main origin of fish by-products.

The main origin of the byproduct derive from trimmings of commercially harvested fish in Norway such as cod, pollock, haddock and others as well as salmon (from aquaculture and wild), herring and mackerel. By- products come from wild caught fish or aquaculture processing.

Processing of fish to different products generate a vast number of by-products. Fillet production contribute with trimmings, skin, backbone, head, liver, gonads and viscera. Gadiform species are also used for clipfish production and saltfish production. The main by-products from clipfish production and salt fish production are mainly heads, viscera, gonads, heads, backbone and swimming bladder. Some studies showed that the product yield for gadiform species for fillets is around 43%, and the rest is by-products (Guerard *et al*, 2005 in Rustad *et al.*, 2007, Kristbergsson *et al.*, 2007) (Fig.1). Other studies showed that production of cod fillets generates 2/3 of the whole body weight as by-products (Slizyte *et al*, 2005, Falch *et al*, 2006a).

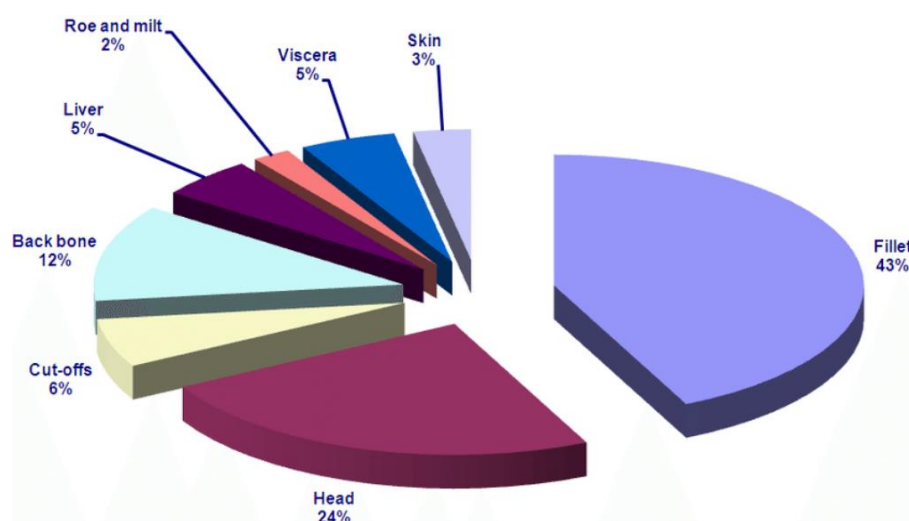


Figure 1 Products and rest raw materials from shore processing of cod (Arason *et al.*, 2009).

The amount of by-products in fish depend on species, size, season and fishing ground (Falch *et al*, 2006a). The data from four gadiform species: *Gadus morhua* (cod), *Pollachius virens* (saithe), *Melanogrammus aeglefinus* (haddock) and *Brosme brosme* (tusk), show that the viscera (all inner fractions) makes up 12–15% of the whole body weight, the heads make 15–20% and the backbone and trimmings make up 18–30% (Falch *et al*, 2006b). An average daily catch of gadiforms from a trawling vessel can produce up to 10 000 kg fillets, which will generate 17000–21000 kg by-products depending on species (Falch *et al*, 2006b)

2.3 By-products: regulation.

The definition, processing and use of animal by-products is harmonized within the EEA (EC 1069/2009 and EC 142/2011) and is ratified in the Norwegian animaliebiproduktforskriften. Animal by-products are placed in three categories, defining how the material should be processed and used. Category 1 is the highest risk category and includes material that should be kept away from any food chain. Category 2 products are also considered as high risk material and include i.a. animal by-products that contain residues of drugs or pollutant as well as products of animal origin not suitable for human consumption. Category 3 is a low-risk material that may be used for feeds for animals that are used for human consumption. Most of this material originate from the parts of animals that are fit for, but not intended for human consumption. If treated correctly, the-byproducts used in aqua feeds, are category 3 by-products.

2.4 Global fisheries and utilization of by-products.

The total fish production (including fish, crustaceans, mollusks and other aquatic animals reached 179 million tons in 2018 (FAO). It is assumed that 35% of the global harvest from fisheries and aquaculture production is either lost or wasted. Around 88% of the total utilized fish production was used for direct human consumption and 12% was used for non-food production. The total global capture fisheries production reached 96,4 million tons in 2018 (FAO). Annual discards from global marine capture fisheries between 2010 and 2014 were accounted for 9.1 million tons, or approximately 10% of the total catch (FAO). It is further estimated that around 11.7 million tons of byproduct is produced globally in processing plants that are not utilized for production of marine ingredients (Jackson *et al.*, 2016). The latter authors also suggested that Asia (excluding China) and Europe have potentially 4.6 million tons and 0.6 million tons unexploited by-products that potentially can be processed into marine ingredients. Approximately 15 % of the global by-products from fisheries is used for human consumption, while the rest is used for the production of fishmeal, silage and animal feed. An indication of the share of raw material from different sources used for production of fishmeal suggested that 19% of fish meal and 26% of fish oil originated from the by-products from the capture fisheries (Fig. 2 og Fig. 3).

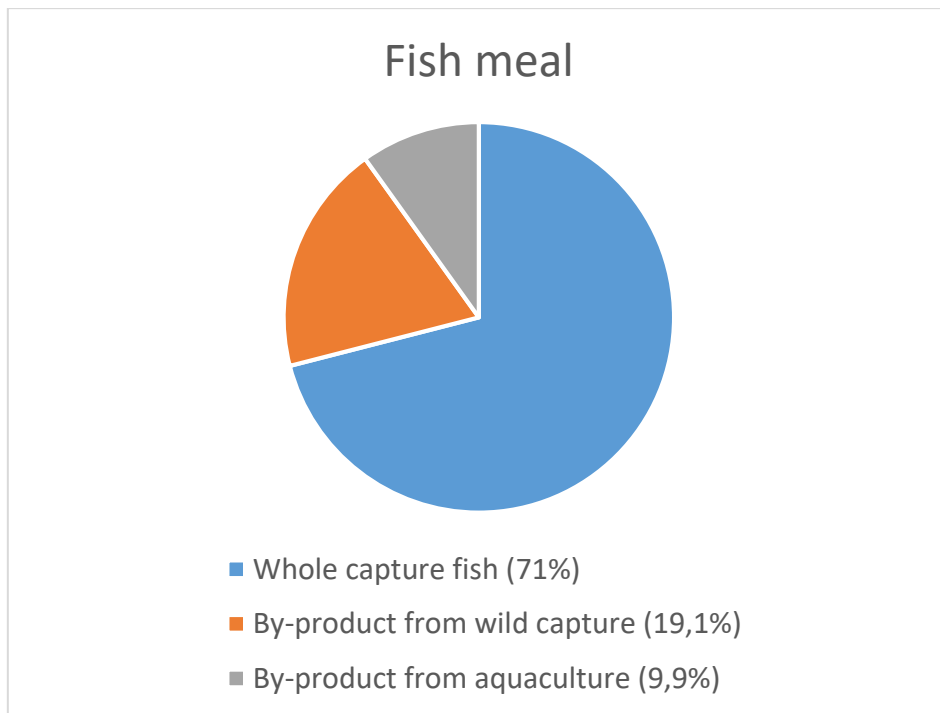


Figure 2 Source of raw material for the production of fishmeal in percentage (Jackson and Newton, 2016)

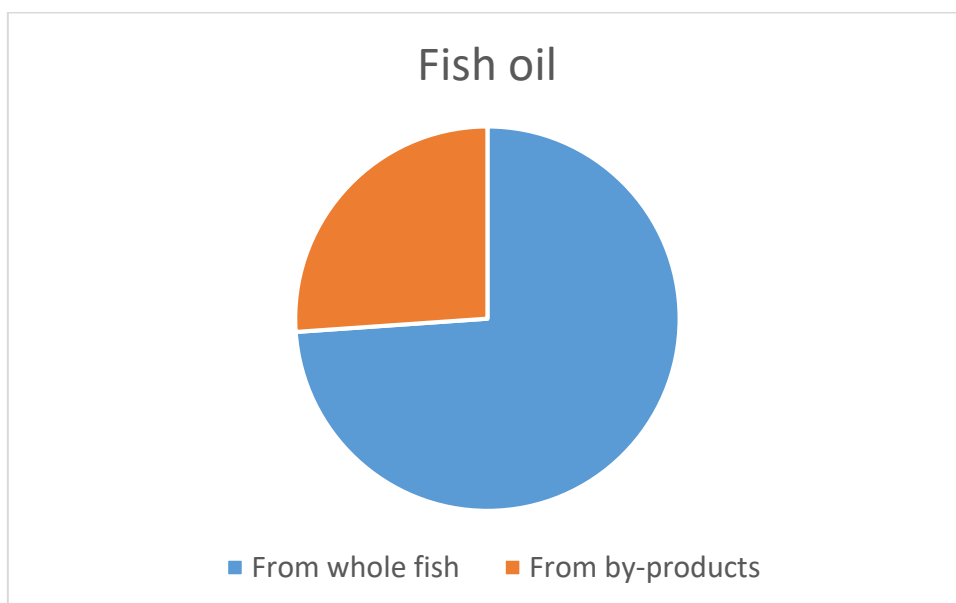


Figure 3 Source of raw material for the production of fish oil in percentage (Jackson and Newton, 2016)

2.5 Fisheries and utilization of by-products in Norway

2.5.1 Fisheries and structure of the fishing fleet

One limitation for full utilization of by-products is the structure of the fishing fleet (Arason *et al.*, 2009). Almost all by-products from onshore slaughtering of fish is already almost fully utilized (Olafsen *et al.*, 2012). The largest potential for increased utilization of by-products is to the by-products generated onboard. These by-products are a larger degree dumped at sea due to inadequate processing facilities and lack of space (Olafsen *et al.*, 2012, Sjømat Norge, 2013).

The Norwegian fleet is complex, so is the fishing pattern. The fleet consisting of a wide range of vessels from small one-man boats to large ocean-going vessels, some of them with fillet fabric onboard. There are 5980 vessels and 11055 fishermen registered in Norway in 2019 (Fiskeridirektoratet 2020). Regulatory, the Norwegian fleet is divided into two groups, coastal fleet and ocean-going fleet. The coastal fleet consists of vessels between 4-5 meter long to vessels with hull capacity under 500 metric tons. Fishing gear such as nets, lines, danish seines are used for fishing and the fish is partially gutted at sea and partially on land. The largest boats in the coastal fishing fleet are often fishing for several days in a row without landing. In this case all fish is gutted at sea.

The ocean fleet can be roughly divided into trawlers, auto liner boats and purse sein boats. Purse sein boats fish on pelagic fish species, while trawl and auto line are mainly used in whitefish fisheries. Most of catch on a trawler are headed and gutted. A number of trawlers have a fillet production on board, these types of trawler generate trimmings, heads and viscera. Some of the factory trawlers have fishmeal and fish oil production on board, where all of the by-products are processed. Of all the fleet groups, the ocean- going fleet utilized less by-products, while the coastal fleet only discard 9 700 tonn of the total of 176 000 tonn in 2018 (SINTEF, 2020).

The main target species are herring, cod, capelin, mackerel, saithe, blue whiting, and haddock. The pelagic fleet catches herring and mackerel for human consumption, and blue whiting, sand eel and Norway pout that are used in production of fishmeal and fish oil either on land or on board the vessels. Most of the fish used for human consumption, is delivered for processing and freezing on land, some vessels have fillet production on board. The by-products from fillet production are either used for fish meal or oil or in the production of silage concentrate.

2.5.2 Utilization of by-products.

In 2018 around 960 000 metric tons of by-products were generated from the total amount of 3,6 mill ton fish and mollusks, caught and produced in Norway or by Norwegian vessels (SINTEF, 2020). Almost 84% was exploited. According to the statistics, the pelagic and aquaculture sectors utilize 100% of the available by-products. The gadiform fisheries are not yet using all the potential, however, the utilization degree has increased from 50% in 2017 to 61% in 2019. 320 000 ton by-products originated from the gadiform fisheries (SINTEF, 2019). The total amount of utilized by-products was 181 000 ton, while the amount of unutilized by-products was 116 400 ton. Approximately 13% of by-product are used for direct human consume, 72% are used as feed for fish and animals, whereby 57% are used in aquaculture feed for salmon and other marine species. By-products used for bio-energy amounts to approximately 15% (SINTEF, 2020).

2.6 By-products: chemical composition.

The nutrient content of fish meal depends on the type of raw materials and manufacturing process used in the production. In general, fish meal produced from whole fish contains 66-74% crude protein, 8-11% crude lipids, and < 12% ash (Hua *et al*, 2019). Fish meal produced from by-products has a somewhat different composition and may contain 52-67% crude protein, 7-14% crude lipids and 12-23% ash (Hua *et al*, 2019). Fish meal produced from byproducts derived from gadiform species, contains 60-67% crude protein, 7-11% crude lipids and 21-23% ash. The protein contents in by-products is slightly lower than in the whole fish, due to less muscle and more bones in the product. Gadiform fishes are generally lean, and the lipid content is therefor in the lower range.

2.6.1 Dry matter, crude protein and amino acid composition.

The role that proteins and amino acids play in the structure and metabolism is critical for all living organisms. The minimum protein requirement for Atlantic salmon is estimated to be between 34% and 48% of the feed, depending on the life stage (age and size). Protein requirement decrease with increasing fish size (NRC, 2011). The protein contents in by-products is significantly higher than the minimum requirements. Twenty primary amino acids

are used by cells in protein biosynthesis, ten of them are essential, as they cannot be synthesized by the fish itself and must be acquired through the diet. By-products contain all the 20 amino acids and the amino acid pattern is relatively similar to fish meal from anchovy (Table 1).

Lysine (Lys) is considered to be the first limiting amino acid (AA) in fish as in higher vertebrates (Abboudi *et al.*, 2006). Content of lysine (7.3%) of crude protein (CP) is higher than the requirement of this amino acid for Atlantic salmon, which is 5.0% of CP (Espe *et al.*, 2007 in NRC, 2011).

The level of methionine in fish meal from by-products is twice the amount in soy protein concentrate, but slightly lower, than in fish meal from anchovy. Nevertheless, the content of methionine (2.71% of CP) is higher than the minimum requirement reported for Atlantic salmon, 1.73% of CP (Espe *et al.*, 2008 in NRC, 2011). Methionine is proved to be the first limiting amino acid in many fish diets, containing high levels of plant protein sources (Goff *et al.*, 2004, Mai *et al.*, 2006, Gatlin *et al.*, 2007, Savolainen, 2010, Jirsa *et al.*, 2013). Low dietary levels of methionine have been shown to suppress growth of marine species (Wilson, 2002, Gatlin *et al.*, 2007).

The level of histidin (2.16% of CP) is lower than soyprotein concentrate and fish meal from anchovy, but is covering the minimum requirement of salmonids (NRC, 2011). Histidin is not only an essential amino acid important for growth, but it is also important for the prevention of cataract in Atlantic salmon (*Salmo salar* L.) smolts (Breck *et al.*, 2005, Waagbø *et al.*, 2010).

Table 1 Crude protein and amino acid composition of fish meal from anchovy, fish meal from white fish by-products, and soy protein concentrate (as-fed basis) (NRC, 2011).

	Fish meal anchovy, mechanically extracted	Soy concentrate	Fish meal white, by-products
Dry matter (%)	92	92	92
Crude protein (%)	65.40	63.63	62.00
Arg	3.68	4.64	4.02
His	1.56	1.58	1.34
Iso	3.06	2.94	2.72
Leu	5.00	4.92	4.36
Lys	5.11	3.93	4.53
Met	1.95	0.81	1.68
Cys	0.61	0.89	0.75
Phe	2.66	3.28	2.28
Tyr	2.15	2.30	1.83
Thre	2.82	2.47	2.57
Tryp	0.76	0.84	0.67
Val	3.51	3.06	3.02

2.6.2 Lipid content.

Studies on lipid content and composition in by-products from gadiform species have shown, that these species store their lipids mainly in the liver, and the lipid depot in this organ contained between 54 and 69% of lipids in cod, saithe and haddock and 43% in tusk (Falch *et al*, 2006a). The viscera and trimmings contain lower amount of total lipids, ranging from 2 to 9% in viscera and as low as 1% in trimmings. The head, trimmings, backbone and viscera that compose more than 60% of the available by-products, accounts only for approximately 1% of the available lipids (240 kg lipids from an average daily catch) (Falch *et al.*, 2006a). Liver makes between 4-6% of round weight in cod, haddock and saithe (Falch *et al.*, 2006b). Lipids stored in the liver are primarily triacylglycerols (90%), while muscle and gonads contain phospholipids with higher levels of polyunsaturated fatty acids (PUFAs) (Falch *et al*, 2006a). According to Falch, polyunsaturated fatty acids (PUFAs) made up 21-45% and 34-63% of the total fatty acids in liver and viscera (Falch *et al*, 2006a)..

2.6.3 Vitamins and minerals.

Fish meal from by-products is generally a good source of vitamins (Table 2). Vitamin B12 is significantly lower, than in anchovian fish meal, however other B-complex vitamins are comparable. B-vitamins are co-factors in the intermediate metabolism of protein, carbohydrates and lipids, and vitamin B deficiency signs are reduced growth and appetite and accumulation of fat around internal organs and fatty liver (Torstensen *et al*, 2008, Espe *et al*, 2016 in Hemre *et al*. 2016)

Table 2 Vitamin composition of fish meal from white fish by-products, presscake meal, fish meal, anchovy, mechanically extracted, and soy protein concentrate (NRC, 2011).

	Fish meal. By-product	Fish meal, anchovy	Soy protein concentr
Biotin	0.08	0.23	0.32
Choline	3.09	4.40	2.60
Folacin	0.35	0.20	0.60
Niacin	59.00	100.00	28.00
Pantothenic acid	9.90	15.00	16.30
Vit B6	5.92	4.64	6.00
Riboflavin	9.10	7.10	2.90
Thiamin	1.70	0.10	6.00
Vit A	-	-	-
Vit B	-	-	-

Vit B12	89.50	352	-
Vit E	8.90	5.00	2.40
Vit K	-	2.20	-

Minerals concentration in by-products is comparable to those of soyprotein concentrate and fish meal anchovy, except concentrations of calcium and phosphorus (Table 3). High amounts of Ca and P regress the quality of ingredient, as they are assumed to reduce the bioavailability of certain trace elements, particularly zinc (Olsen *et al.*, 2019).

Table 3 Mineral composition of fish meal from white fish by-products, presscake meal, fish meal, anchovy, mechanically extracted, and soybean protein concentrate (NRC, 2011).

	Fish meal. By-product	Fish meal, anchovy	Soy protein concentrate
Dry matter (%)	91	92	92
Calcium	7.31	3.73	0.30
Phosphorus	3.81	2.43	0.65
Sodium	0.78	1.10	0.04
Chlorine	0.50	1.00	0.04
Pottasium	0.83	0.90	2.11
Magnesium	0.18	0.24	0.29
Sulfur	0.48	0.54	0.42
Copper	5.90	9.03	23.00
Iron	181.00	220.00	140.00
Manganese	12.40	9.50	30.60
Selenium	1.62	1.36	0.10
Zinc	90.00	103.00	52.00

2.7 Challenges.

2.7.1 Technological constrains.

Stable and sustainable supply is deemed necessary for continuous and profitable production of ingredients from by-products (Thorkelsson *et al.*, 2009). The by-products are landed in many places along the coast, and good logistics and technological solutions must be developed - both aboard and on land. Seasonality of the catches is also a factor that need to be considered and that can be problematic in planning large-scale production of proteins and peptides.

Raw material and correct handling of the raw materials are crucial for the quality the final product. To ensure high quality, it is important to process by-products immediately after

production, and it is advantageous to start production on-board the fishing vessel. Lack of space, lack of optimal conservation and not enough manpower on-board most fishing vessels make this alternative challenging. If there is no space for handling the by-products onboard the vessel, the production have to take place on land. Akse et al. (2002) showed that cod could be landed ungutted and gutted on shore within 12-48 hours after catch, depending on the season, without reducing the quality of the fish or the by-products. Mature (spawning) cod could be stored ungutted for up to 48 hours, while feeding cod, so-called capelin cod, should be gutted within 12 hours. This will allow collection of the by-products from the on-shore landing facilities and transportation to processing plants (Grimsmo *et al.*, 2009). However, only the vessels, fishing close to the shore, are capable of delivering by-products within 12 hours.

2.7.2 Biochemical constrains.

By-products that includes parts of fish with high enzymatic activity, i.e. gastrointestinal tract, containing highly active digestive enzymes. Different rapid autolysis and liquification of the by-product (Rustad *et al.*, 2011). Due to the fact that the enzymatic activity in the gastrointestinal tract is relatively high, the lipids in the by-products are susceptible to fast degradation even at low storage temperatures (Rustad *et al.*, 2011). Proteolysis lead to break down of proteins into amino acids, while lipases and phospholipases lead to the formation of free fatty acids, which reduces sensory quality and oxidative stability of fat. Some by-products, especially those from fat fish, contain high amounts of oil that is highly susceptible for oxidation because of the polyunsaturated fatty acids. Intestines and gills have high bacterial numbers, and are highly susceptible to microbial spoilage. In addition, the microbial flora on equipment and people handling the fish and by-products may also introduce harmful microorganisms. To ensure high quality of product, it is important to separate easily degradable parts from the more stable fractions and treat these with special care. Hygienic handling of the raw material is a key for high quality of the products and a key for successful utilization and processing of by-products (Thorkelsson *et al.*, 2009).

2.7.3 Ethical consideration

Large amount of fish by-products are currently not utilized. From an ethical aspect this is not acceptable and by-products can also add value to fisheries. At present, the total cost of bringing ingredients to shore may exceed the expected market price (Grimsmo *et al.*, 2015). The development is however positive, and the utilization of by-products is increasing. Utilization of by-products from the pelagic fisheries and aquaculture has more or less reached the maximum, while contribution from the white fish fisheries is constantly increasing. To ensure better use of fish by-products, it is important to develop technologies to store and keep the products, and the valuable components, as well as to encourage the stakeholders and focus on the market.

3. *Calanus finmarchicus*

Zooplankton is trophic level between the primary producers (phytoplankton) and organisms on higher trophic levels in the marine food web, including marine fish and marine mammals. The Norwegian seafood federation, Norway's largest association for seafood industry, has in its vision "Seafood 2030 - a blue change of pace" declared that in order to reduce environmental impact of seafood industry, a significant share of raw materials for seafood production should come from the lower trophic level, including marine algae, zooplankton and phytoplankton, (Sjømat Norge, 2017). One zooplankton species of economical interest is *Calanus finmarchicus*. Since 2019, the Norwegian government is allowing commercial harvesting of *C. finmarchicus* after several years issuing only research concessions.

3.1 *Calanus spp*

The *Calanus* species, *C. finmarchicus*, *C. glacialis* and *C. hyperboreus* are the most important species and the prime herbivores in the Arctic and northern seas (Tande, 1991, Weydmann *et al.*, 2008, Søreide *et al.* 2008). The three species are distributed in Arctic waters, including the Norwegian Sea, the Barents Sea, the White Sea, the Arctic Ocean, the Greenland Sea and the coastal waters bordering Siberia, East Canada and Alaska. They have different and distinct centers for over-wintering (Hirche *et al.*, 1996, Falk-Petersen *et al.*, 2009).

3.2 *Calanus finmarchicus*

C. finmarchicus is the dominant *Calanus* species in the Norwegian Sea and is one of the largest marine resources in the entire Northeast Atlantic. Stock assessment indicates that a standing population of *C. finmarchicus* alone is approximately 70-80 million tons in the Nordic Seas (Melle *et al.*, 2014). The biomass estimate is however, highly uncertain, as the population numbers vary considerably between the years and throughout the year. Despite the uncertainty, it has been recognized for a long time, that *C. finmarchicus* has a significant potential for harvesting and value creation. It is assumed that *Calanus spp* in Norwegian Sea, Icelandic sea and Greenland sea have a production potential between 120-500 million tons wet weight per year (Tokle, 2008). Thus the potential for utilization is significant, and according to the results

of the Calanus program, carried out from 2000 to 2005, harvesting of Calanus is technically possible. Research concessions have been used to collect scientific proof that commercial fisheries based on strict regulation and quotas, will not have a negative effect on the stock of *C. finmarchicus*, or other marine resources. Some studies even point to no effect or even positive effects harvesting this plankton. A data stimulation suggests that withdrawal of 10% of the biomass would lead to double increase in the total biomass in the following generation and that even 50% extraction will not have a decisive effect on subsequent generations (Slagstad *et al.*, 2005, Tokle, 2008).

Zooplankton plays a key role in the lipid-based energy flux in the Arctic. It converts low-energy carbohydrates and proteins in ice algae and phytoplankton into high-energy lipid compounds, which are then transferred through the food chain as the major source of energy for the large stocks of fish, birds and marine mammals in the Arctic (Lee, 1971, Sargent *et al.* 1986, Falk-Petersen *et al.* 1990, 2000, Lee *et al.* 2006). It is assumed that only 10–20% of the energy transferred between each trophic level is converted to biomass at the next level (Parsons *et al.*, 1988). Thus, a sustainable fishing at lower trophic levels, is claimed to provide a many-fold gain in potential harvestable biomass (Pitcher, 2008).

3.3 Biology of *C. finmarchicus*

The biology including the lipid composition of *C. finmarchicus* has been a subject for extensive research over the years (reviewed by Sargent *et al.*, 1986, Kattner *et al.*, 1987, Hirche, 1996, Lee *et al.*, 2006, Pedersen *et al.*, 2014). *Calanus finmarchicus* has a one-year life cycle in Barents and the Norwegian Seas. During the life cycle the copepods go through reproduction and development in surface waters before overwintering in diapause at depth. Diapause depth is usually between 400 m and 2000 m in the open ocean and 100 m and 150 m. in coastal habitats (lochs and fjords) (Hirche 1996, Heath *et al.*, 1999, Visser *et al.*, 1999, Kaartvedt 2000, Falk-Petersen, *et al.* 2009).

3.3.1 Life cycle

Calanus spawns in April- May during or just after the phytoplankton bloom peak (Tande *et al.* 1991, Niehoff *et al.*, 2002, Madsen *et al.* 2008, Falk-Petersen *et al.*, 2009) (Figure 4).

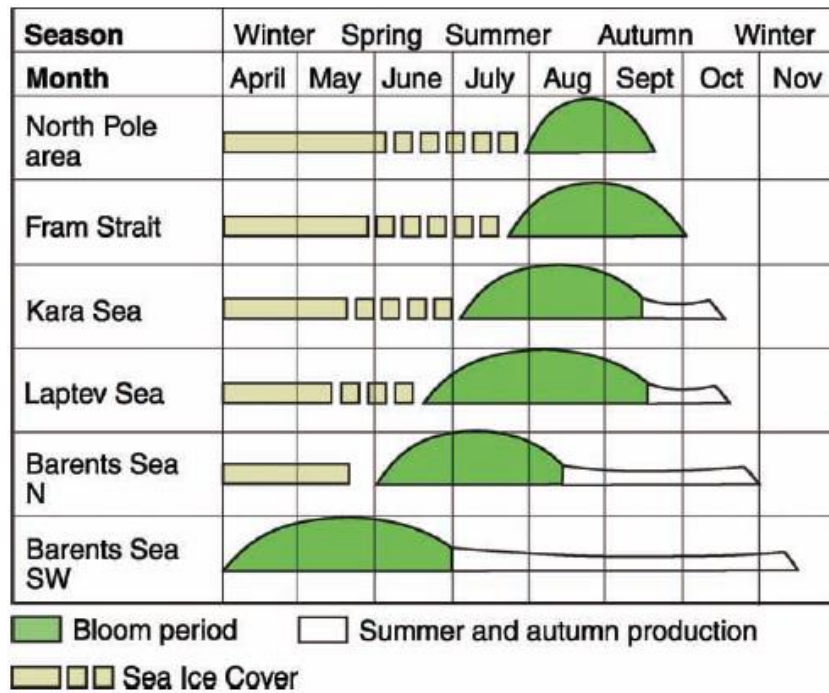


Figure 4 Time-related plankton blooms in the Arctic Oceans (Falk- Petersen et al., 2009)

The new generation develops through six nauplii (N1–N6) and five juvenile copepodite stages (C1–C5) which finally develop into the adult female or male stage (CVIf or CVIm, respectively) (Lee *et al.*, 2006, Falk-Petersen *et al.*, 2009) (Figure 5)

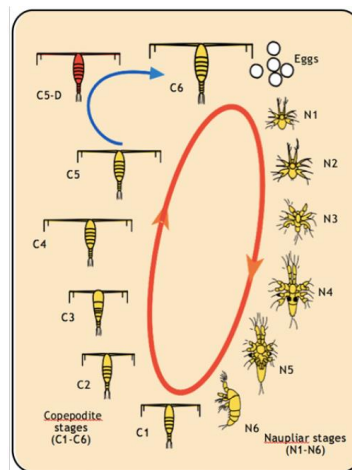


Figure 5 Schematic representation of the Calanus life cycle

The stage I copepodites (1-2.2 mg dry mass (DM)) develop to the lipid-rich over-wintering stage IV (40-70 mg DM) and stage V copepodites (130-240 mg DM) by June- July (Falk-Petersen *et al.*, 2009). The copepodites IV-V descend to deep water in June-July, where they

undergo diapause (Falk- Petersen *et al.*, 2009). The over-wintering stage, mainly stage V copepodites, develops into males and females in January. Females develop ovaries between January and March and during late winter (February- April) the population migrates into the surface waters, ready to spawn. The animals are then transported by surface currents.

In the CIV and especially the CV copepodite stage the copepods accumulate large amounts of lipids, predominantly wax esters stored in a lipid sac (Falk-Petersen *et al.* 2009, Clark *et al.* 2012). Accumulation of wax esters begins in stage IV copepodites in spring-summer and reaches maximal levels in stage V copepodites prior to overwintering (Lee *et al.* 1971, Scott, 2000).

3.3.2 Energy utilization

When descending to deep waters in the autumn, *C. finmarchicus* has gained enough energy to survive during starvation periods up to 9 months as well as to produce gonads by the end of the diapause before ascending to the surface in the spring. The onset of vertical migration is triggered by a high lipid level, and that the copepodites that do not reach the sufficient level during the phytoplankton bloom, molt to females and start a new generation (Irigoien *et al.*, 2004, Clark *et al.* 2012; Bergvik, 2012). Estimations suggest, that only around 5% of the stored lipids are consumed during overwintering (Jonasdottir, 1999). Adult females maintain a considerable amount of lipid when they reach the surface which later is used to produce eggs before the phytoplankton bloom, when food levels are still low.

3.4 Chemical composition of *C. finmarchicus*

C. finmarchicus consist of appr. 80-82% water, 10-11% protein, 5-8% lipids and 2% ash (Utne, 1974, Vang *et al.*, 2013)

3.4.1 Lipids

The lipid content of *C. finmarchicus* depends greatly on the stages of development, season, and location of sampling (Pedersen *et al.*, 2014). Compared to most fish oils and krill oil, which mainly consist of triacylglycerols and phospholipids, the lipids of *C. finmarchicus*

is mainly wax esters (WE), consisting of long-chain monounsaturated fatty alcohols esterified to saturated or unsaturated fatty acids (Pedersen, 2014). *Calanus* use the WE to regulate buoyancy and vertical migration, gonad formation and as a long-term energy store during the diapause (Jonasdóttir *et al.*, 1999; Visser *et al.*, 1999). By changing the density of the WE layer, the *C. finmarchicus* can stay at sea depth during diapause without utilizing energy (Table 4).

Table 4 Lipid class composition of late copepodite stages and adult in C. finmarchicus sampled in different periods and presented as % total lipids Falk-Petersen et al (1987).

% of total lipids			
Lipid class	June	Oktober	March
Triacylglycerols	8.9	1.3	Nd
Sterols	1.2	2.6	3.2
Free fatty acids	0.2	Nd	1.7
Wax esters	85.4	88.1	84.9
Phospolipids	4.2	7.3	10.3

Nd- not detected

It is believed that the WE composition of the copepods reflects both their marine habitat, the relative rates of ingestion and the fatty acid composition of the specific phytoplankton species they feed on (Lee, 1974, Kattner *et al.*, 1987, Falk-Petersen *et al.*, 2009). Triacylglycerols and wax esters reflect the dietary fatty acids, whereas phospholipid fatty acids reflect biosynthetic pathways and membrane structural requirements (Lee, 1974). The total amount of lipids and WE in calanoid copepods depend on the latitude (reviewed by Sargent and Falk-Petersen, 1988, in Pedersen, 2014). The Arctic species contain the highest amounts, due to low water temperature.

The lipid reserves of *C. finmarchicus* copepodite stages IV– V may compose almost 60% of its total dry weight (Kattner *et al.*, 1987, Jónasdóttir *et al.*, 1999, Scott *et al.*, 2000, Lee *et al.*, 2006). The WE content has been found to be as high as 80–90% of the total lipids, while triacylglycerols (TAG), phospolipids (PL), and free fatty acids (FFA) are minor constituents (Table 5).

Table 5 Total lipid and storage lipid class data of *C. finmarchicus* (Lee et al, 2006)

	Total lipid (TL) (% of dry weight)	WE (% of TL)	TAG (% of TL)
Copepodite IV	53	44	10
Copepodite V	34	68	5
Female	24	62	6

The WE of *C. finmarchicus* contain fatty alcohols that are mainly monounsaturated, 20:1n-9 (gondoic acid) and 22:1n-11 (cetoleic acid) may constitute 62 to 93% of the lipids, while the saturated alcohols 14:0 (myristic acid) and 16:0 (palmitic acid) may make up from 8 to 24% (Pedersen *et al.*, 2007, Falk- Petersen *et al.*, 2009). The very high amounts of de novo synthesized 22:1 and 20:1 fatty alcohols and acids is a store of exceptional high energy value lipids (Falk- Petersen *et al.*, 2009). The content of polyunsaturated n-3 fatty acids in the WE may account for 20–30% of the total fatty acids content, dominated by 18:4n-3 and 20:5n-3 (Table 6).

Table 6 Fatty acid composition (%) derived from *C. finmarchicus* oil, FFA and wax esters (Vang, 2015)

Fatty acid	Oil	FFA	Wax ester
14:0	14.7	4.7	15.4
16:0	9.3	21.2	7.6
16:1 n-7	6.7	4.1	6.4
16:2 n-4	1.0	0.6	0.9
16:3 n-4	2.5	1.7	2.4
18:0	1.0	7.9	0.8
18:1 n-9	4.1	2.9	4.0
18:1 n-7	2.5	1.8	2.4
18:2 n-6	0.9	1.1	0.8
18:3 n-3	1.3	1.2	1.1
18:4 n-3	12.4	8.0	11.4
20:1 n-9	5.9	1.3	6.0
22:1 n-9	9.0	1.7	8.8
20:5 n-3	14.4	17.0	11.6
24:0	0.7	0.5	0.6
24:1 n-9	0.8	0.6	0.6
22:6 n-3	7.7	18.3	4.1

Σ SAT	25.7	34.3	24.4
Σ MUFA	44.1	30.0	40.4
Σ PUFA	40.2	48.0	32.2
n-3	35.8	44.6	28.1
n-6	0.9	1.1	0.8

The fatty acid composition of TAG in *C. finmarchicus* lipids are similar to the pattern found in TAG of fish oils (McGill *et al*, 1992 in Pedersen *et al.*, 2014). The PL of *C. finmarchicus* are also characterized by a very high content of n-3 fatty acids (Table 7). The amount of EPA and DHA in PL of *C. finmarchicus* is close to the amount of EPA (23.65–28.10%) and DHA (16.71–21.03%) present in the PL in oil extracted from krill. Some studies indicated that bioavailability of EPA and DHA is higher, when these form of n-3 PUFA appear in the form of PL, making, for example, krill oil superior to fish oil that contains EPA and DHA as TAG (Rossmeisl *et al.*, 2012, Ulven *et al.*, 2011 in Xie *et al.*, 2018).

Table 7 Fatty acid composition (mass %) of triacylglycerol and phospholipids in C. finmarchicus, late copepodite stages and adults. Source Albers et al (1996), Fraser et al (1989) (in Pedersen et al, 2014)

Fatty acids	Triacylglycerol		Phospholipids	
	June-August ¹	March ²	June- august ¹	March ²
20:5n-3	8.7	7.4	19.2	23.1
22:6n-3	5.8	5.1	37.4	30.9
Σ SFA	52.7	30.9	32.7	24.5
Σ MUFA	18.1	28.7	5	2.8
Σ n-3 PUFA	23.9	26.1	59.9	59.1

A significant amount of stearidonic acid (SDA, 18:4 n-3) is also found in addition to EPA and DHA. Stearidonic acid is an intermediate metabolite between α -linolenic acid (ALA) and EPA in the n-3 biosynthetic pathway and is assumed to be a superior precursor to EPA compared to ALA, because SDA is not dependent on the rate limiting enzyme Δ 6 desaturase, in the conversion from ALA to the EPA (Calder *et al.*, 2013; Lenihan-Geels *et al.*, 2013).

The n-3 PUFA may account for 40 – 45 % of total fatty acids in *C. finmarchicus* late copepodite stages CIV and CV (Kattner *et al.*, 1987, Bogevik *et al.*, 2009). Still, the level of 20:5-n3 and

22:6 -n3 increases from approximately 13 to 20% and 10 to 30- 40% respectively between copepodite stage V and females (Falk- Petersen *et al*, 2009).

3.4.2 Astaxantin

Astaxanthin is the most commonly occurring carotenoid in copepods, it may contribute to as much as 85–90% of the total pigment (Pedersen, 2007). Studies of copepod carotenoids have shown concentrations ranging from 550–850 µg/g based on dry weight (Lotocka *et al*, 2001; Lotocka *et al.*, 2004). It is believed that one of the central functions of astaxanthin (esters) in calanoid copepods is antioxidant protection of storage lipids. The common know name of *C. finmarchicus* is “red feed” which reflects its the red color. The characteristic coloration is due to the large quantity of lipophilic carotenoid pigments, and Calanus-oil contain 1500 µg/g of carotenoids, which is a considerably higher content, compared to other natural crustacean sources. High content of carotenoid make Calanus-oil a excellent natural alternative to synthetic astaxanthin as feed ingredients to organic farmed salmon (Hynes *et al*, 2009).

3.4.3 Protein and amino acids composition

In general copepods have a high protein content (44-52% DM) and a favourable amino acid profile. All 20 amino acids are present in *C. finmarchicus* (Wiborg, 1974). The table below represent the typical amino acid profile of *C. finmarchicus*, analyzed in Mai 1973 and of the commercially available product called Calanus ® Powder: Aqua, produced by Calanus AS (Table 8).

Table 8 Amino acid content in *C. finmarchicus* (g/ 100g⁻¹ protein).

	Amino acid content in <i>C. finmarchicus</i> (Wiborg, 1974)	Calanus ® Powder: Aqua, produced by Calanus AS.
Arginine	7.11	5.96
Histidine	1.92	2.54
Isoleucine	3.72	5.41
Leucine	5.92	8.05
Lysine	6.67	6.80
Methionine	2.28	3.06
Phenylalanine	2.88	5.15
Threonine	3.25	4.99
Tryptophan	1.01	1.99
Valine	6.17	6.15

Alanine	5.85	6.31
Aspartic acid	7.37	9.44
½ cystine	0.71	1.64
Glutamic acid	10.20	12.18
Glycine	6.89	6.04
Proline	2.94	4.01
Serine	3.11	4.43
Tyrosine	3.70	5.83

C. finmarchicus contain a well balanced profile of essential amino acids, that suits the requirements of Atlantic salmon (*S. salar*)

3.5 Harvesting of *C. finmarchicus*

Since the end of the 1950s, small amounts of *C. finmarchicus* have been harvested in Norway (Larsen, 2009). During the 1990s, there was a growing interest in *Calanus spp* as a potential feed ingredient in aqua feed for the fast growing aquaculture industry. In 2006, the Ministry of Trade and Fisheries issued the regulation, prohibiting the Norwegians vessels to fish and land calanus, krill and zooplankton in the North-East Atlantic, ICES zones I – XIV. It was decided, that a thorough evaluation of stocks and ecological consequences was needed before a commercial harvest of zooplankton could be initiated.

In the period 2003-2007, the Norwegian Directorate of Fisheries granted permission to conduct experimental fishing of up to 100 tons of *C. finmarchicus* (wet weight) every year. During this period, between 6 and 98 tons were harvested annually (Fiskeridirektoratet, 2020). In 2008 a Tromsø- based company Calanus AS received a five year permit to carry out a research based harvest of up to 1,000 tons of this zooplankton per year. The fishing operation could be conducted in the areas from the Norwegian baseline within Norwegian economic zone and in the fishing area around Jan Mayen (Figure 6). The annual catch between 2008 and 2012 varied between 27 tons and 133 tons, and the fishing took place mainly in the coastal waters.

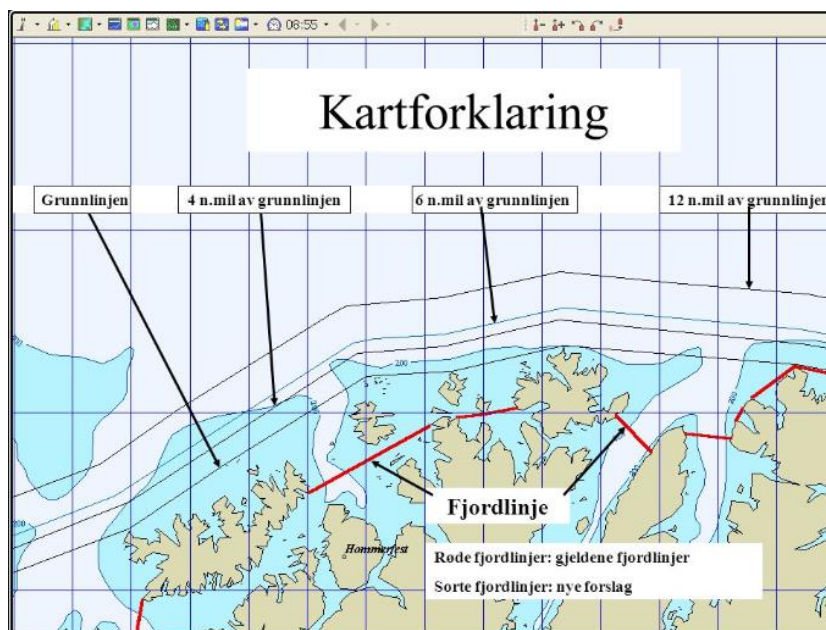


Figure 6 Map of the coastal waters, defining the fjordline, baseline and other lines.

In 2013 a new 5 year permission was issued to Calanus AS. The company was given permission of harvesting 100 tons of the annual quota in areas between the baselines and the fjord lines. In 2014, the permit to conduct fishing in the coastal waters was extended to 500 tons (Table 9 and figure 7).

Table 9 Catch pr year (Råfisklaget register)

Year	Catch (t)	Year	Catch (t)
2003	17	2012	133
2004	6,5	2013	110
2005	52,5	2014	280
2006	98	2015	513
2007	70	2016	649
2008	88	2017	760
2009	-	2018	1362
2010	27	2019	352
2011	128	2020 (per 01.10)	-

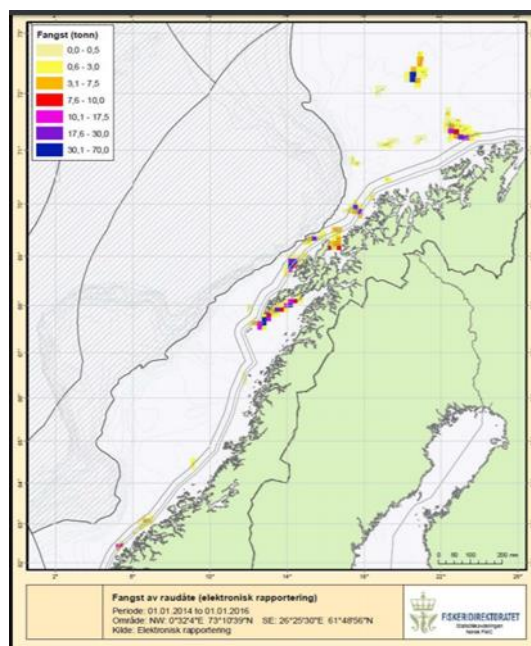


Figure 7 The locations of harvesting

3.6 New regulation regime

During all these years of experimental harvesting *C. finmarchicus*, the participants had to collect and report the information on the distribution and composition of the catches, by-catch of fish eggs, larvae and undersized fish. Based on the scientific research and the information collected from the test fishing, the Directorate of fisheries in cooperation with the Marine research institute, formulated a management plan for *C. finmarchus* and opened for commercial fishing in 2019. The catch quota was set at 254 000 tons for harvesting in Norwegian economic zone (NØS) north of 62 ° N, west of 24 ° E and beyond 12 nautical miles (nm) in the Jan Mayen zone. Limited permissions for harvesting 3000 tons in the coastal zone, were granted to ten participants, divided into a category 1, assigned to corporations with no active fishermen, according to the Participant Act §6, and a category 2, assigned to corporations owned by active fishermen. When allocating permissions in the category 1, the Directorate of Fisheries prioritized the actors who would facilitate further processing either on land in Norway or on board the harvesting vessel. Additionally, Calanus AS was assigned a permit to harvest 5,000 tons of *C. finmarchicus* annually until 2022 in coastal areas.

3.7 Challenges.

3.7.1 Public acceptance

However, opening of the commercial harvesting of *C. finmarchicus* in 2019 has been a highly disputed and criticized decision. *C. finmarchicus* throughout its whole life cycle is the main food for fish larvae and fish fry in addition to adult pelagic fish. It is also the main food for Norwegian spring-spawning herring. (Tiller, 2008). Depletion or overfishing in the low trophic level of the food web may have severe negative effects on the ecosystem. By-catch of eggs, larvae and fry of various fish species is also mentioned as a significant negative effect of the *Calanus* harvesting. Trawling over the spawning grounds or larvae drift, will inevitably lead to a certain degree of by-catch of fish larvae and eggs. Whether these arguments are scientifically justified or not, the public acceptance is anyway crucial for development use of *Calanus sp* as feed ingredients or functional food for humans. The available data suggest that consumers willingness to accept new products is negatively affected by fear if a product can cause physical damage or represent a threat to biodiversity (Gaivoronskaia, 2008). Public acceptance play a significant role in the development of markets for *C. finmarchicus*.

3.7.2 Technological limitations

Ten permissions for harvesting 3000 tonn of *C. finmarchicus* in the coastal zone have been issued since opening of the commercial harvesting in 2019. Only one of those have been activated. A limiting factor that might explain the slow start of commercial fisheries is the current harvesting technology. The current technology is limited to harvesting on surface-dwelling stages, which is significantly spread in the surface, making trawling complicated and time-consuming. Harvesting of *C. finmarchicus* is carried out with a pelagic trawl, and the harvest methods are based on filtration of water through very fine meshed nets, with mesh size of 0.5 mm (0.75 mm og 1.08 mm) (Larsen, 2009). The period of catch is limited to a few summer and early autumn months, when the copepods have entered stage CIV-CV, containing most lipids.

A new technology of harvesting *Calanus sp* by bubble-induced upwelling, was tested in 2011. The idea of bubble flotation-enhanced harvesting is to bring the target species up to the ocean surface with the help of air bubbles. Concentrating the copepods at the surface enables a more

energy efficient method for harvesting and reduce the by-catch of other marine organisms (Grimaldo *et al.*, 2011). This technology is still being tested and not yet available for commercial use.

Onboard handling is another challenging factor. *C. finmarchicus* and other zooplankton species contain endogenous enzymes that cause autolysis rapidly after death if they are not inactivated. Several studies have shown that the enzymes are active even at moderate freezing temperatures (-20 to -15° C), leading to enzymatic degradation of lipids and formation of FFA during long time storage (Kolakowska, 1986, Ohman, 1996, Bergvik *et al.*, 2012). Rapid freezing and storage below -70°C or immediate lipid extraction by solvents are the methods that are suggested to avoid enzymatic hydrolysis of lipids in *Calanus sp.* (Ohman, 1996). Another alternative to inactivate the autolytic enzymes by heating immediately after the harvest has been suggested (Bergvik *et al.*, 2012). None of these methods are however applicable on board the fishing vessels currently employed in the harvest of *C. finmarchicus*. A short storage time at -20°C from harvest until processing might reduce levels of FFA. This alternative press the fishing area closer to the shore, which is unfavorable from the ecological and resource management point of view. A future plankton fishery will thus require vessels with the ability to instantly freeze or process the plankton to prevent it from deteriorating.

3.7.3 Nutritional limitations

As earlier mentioned, the lipids of *C. finmarchicus* appear mostly as wax esters (WE), unlike fish oils and krill oil, that main lipid classes are TAG and phospholipids respectively. WE are generally more hydrophobic than TAG and are therefore more difficult to emulsify during the digestion process (Bogevik, 2011). Pelagic fish such as herring, sardines and anchovies are directly consuming zooplankton and are evolutionarily adapted to digest the WE and to absorb free fatty alcohols to a large extent (Sargent *et al.*, 1979, Place, 1992). Atlantic salmon (*Salmo salar*) is also able to adapt to increased dietary intake of WE from *C. finmarchicus* by increasing bile production and lipolysis activity (Bogevik *et al.*, 2009). It can tolerate up to a 37,5% inclusion of WE in feed oil, while higher inclusion results in reduced digestibility and growth (Olsen *et al.*, 2004, Bogevik, 2011).

4. Mesopelagic fish.

Small species of fish, living in the twilight zone in the deep waters, are the organisms that most likely dominate the world's total fishes biomass. Despite enormous numbers, this species remain one of the least investigated components of the open-ocean system. It constitutes a large potential resource, which has so far been little utilized. There has however been a growing interest in these species lately, as apparently this biomass can contribute to the long term Blue Growth initiative set by the European Union, i.e., “sustainably unlocking the potential of seas and oceans.” (FAO, 2020). Although uncertain to what extent mesopelagic species is a suitable food for humans, these species still represent an interesting feed resource and they might be a very interesting new resource for the fishmeal and fish oil industry.

4.1 Mesopelagic zone, mesopelagic fish- habitat and biomass.

The mesopelagic zone (twilight zone) is a zone between 200 and 1000 meters. It makes up about 60% of the earth's surface and 20% of the ocean's volume, constituting a large part of the total biosphere (IMR, 2017). Mesopelagic fish, small fish, living in the twilight zone, play an important role in oceanic energy dynamics. They are predators of zooplankton (Gjørseter *et al.*, 1980, Choy *et al.*, 2013) and prey for fish (Olaso *et al.*, 2005), seabirds (Petry *et al.*, 2007), and marine mammals (Goetsch *et al.*, 2018, Gimenes *et al.*, 2018, Pusineri *et al.*, 2018). Mesopelagic fish move upwards to the epipelagic zone to feed on drifting zooplankton during the night and thereafter migrate down several hundred meters to their daytime depths (Salvanes *et al.*, 2001, Klevjer *et al.*, 2020). This migration, called "diel vertical migration", acts as a pathway for carbon transport. Fish actively transport organic and inorganic carbon from the surface waters by metabolizing the surface-derived food and transform it in deeper ocean layers via mortality, faeces and respiration of CO₂ (Davison *et al.*, 2013). This migration, constitutes the largest vertebrate migration in the biosphere due to the substantial biomass of this fish (Irigoien *et al.*, 2014). Without this pump, the partial pressure of atmospheric CO₂ would be twice its current value (FAO, 2020). Most mesopelagic species have developed several adaptations to a life under low light intensity: sensitive eyes, dark backs, silvery sides, ventral light organs, and reduced metabolic rates for deeper living fish.

They are abundant along the continental shelf in the Atlantic, Pacific, and Indian Oceans and in deep fiords, and are less abundant offshore and in Arctic and sub-Arctic waters. Some species are distributed worldwide, and many are circumpolar, especially in the Southern Hemisphere (Salvanes *et al*, 2001). The mesopelagic zone is the habitat of approximately seven hundred species.

Gjørseter and Kawaguchi (1980) and Lam and Pauly (2005) suggested that it was approximately 1000 million tonnes of mesopelagic biomass. Recently, the biomass was calculated to 10,000 million tonnes, 10 times more than first estimated (Irigoien *et al.*, 2014). This number is equivalent to 100 times the annual catch of traditional fisheries (FAO, 2018). It is at the same time suggested, that most of the gear used to obtain the available information might underestimate the present biomass, and that the numbers are considerably higher (Gjørseter *et al*, 1980, Kaartvedt *et al.*, 2012, Irigoien *et al*, 2014)

A number of studies carried out in the Norwegian sea, have shown that considerable nutrient-dense biomass can be found there (Haug *et al.*, 2017, Olsen *et al.*, 2019). The species variety is low, and the biomass consists of mainly six species: two species of mesopelagic fish, the glacier lanternfish (*Benthosema glaciale*), and the silvery lightfish (*Maurolicus muelleri*); the decapod *Eusergestes arcticus*; the decapod genus *Pasiphaea*; the euphausiid Northern krill (*Meganyctiphanes norvegica*) and the scyphozoan helmet jellyfish (*Periphylla periphylla*) (Olsen *et al.*, 2019, Wiech *et al.*, 2020). The largest stocks of mesopelagic fish in the Norwegian Sea are northern spotted fish (*Benthosema glaciale*) and salmon herring (*Maurolicus muelleri*) (Alvheim *et al.*, 2020, Wiech *et al.*, 2020). The continuation of the presentation of mesopelagic fish will be focused on these two species.

In the Norwegian Sea and fjords, the *M. muelleri* forms acoustically visible scattering layers (SLs), while the deeper living *B. glaciale* appears acoustically more scattered and less dense (Standal *et al.*, 2020). Distribution of both species depend on seasonally changing variables including light, temperature, salinity, food abundance and piscivorous predators (Grimaldo *et al.*, 2020).

4.2 Biology.

4.2.1 Northern spotted fish (*Benthoosema glaciale*).



Figure 8 Northern spotted fish (*Benthoosema glaciale*).

Northern spotted fish or glacier lantern fish, is the most common species of lanternfish and an important part of mesopelagic ecosystem in northern North Atlantic (Catul *et al.*, 2010). It reside at depth between 375-800 m during daytime and 12-200 m during night. This species range between 2- 5.5 cm in length, but can be up to 10,3 cm. The main prey is calanoid copepods, amphipods and euphasiids. One of the important characteristic of the lanternfish is its glowing effect due to the presence of the photophores systematically arranged on their body (Catul *et al*, 2011).

4.2.2 Salmon herring (*Maurolicus muelleri*)

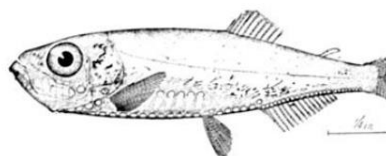


Figure 9 Salmon herring (*Maurolicus muelleri*)

Salmon herring or Mueller's pearlside silvery lightfish is a bathypelagic marine fish, found in the depth range 251-1524 m, but usually between 300-400 m. Common length is 4 cm, can be up to 8 cm. It is a cyclic selective feeder of copepods and euphasiids.

4.3 Chemical composition.

Mesopelagic fish represents a large standing biomass but is the least investigated biomasses both in terms of distribution and abundance, as well as the nutrient content and nutritional value.

Recent research on mesopelagic species in the Norwegian sea are carried out to study the chemical composition. Analysis have shown that several of the mesopelagic species were nutrient-dense, containing high levels of vitamin A1, calcium, selenium, iodine, EPA and DHA (Olsen *et al.*, 2019, Alvheim *et al.*, 2020), however the level of wax esters and a concentration of a heavy metal cadmium in *B. glaciale* might be a challenge (Olsen *et al.*, 2019, Wiech *et al.*, 2020).

A number of studies on chemical composition of *B. glaciale* and *M. muelleri* showed that mesopelagic species are rich in lipids and protein (Alvheim *et al.*, 2020, Grimaldo *et al.*, 2020). Most of the nutrients analyzed are similar to or higher than those for herring (*C. harengus*) and blue whiting (*M. poutassou*) commonly used to produce fishmeal and fish oil for the aquaculture industry (Table 10).

Table 10 Chemical composition of protein, total fat and dry matter in two mesopelagic fish species caught in three fjords in western Norway (mean \pm SD), herring and blue whiting (Alvheim *et al.*, 2020; Sathivel *et al.* 2003, Derkach *et al.*, 2017)

	Protein g/100g	Total fat g/100 g	Dry matter %
<i>M. muelleri</i>	12.3 \pm 0.4	17.8 \pm 8.1	33.3 \pm 8.1
<i>B. galciale</i>	14.0 \pm 0.5	13.7 \pm 3.7	30.8 \pm 3.9
<i>C. harengus</i>	14.5	8.8	26.1
<i>M. poutassou</i>	16.9-17.5	2.7-4.2	22.3-24.0

4.3.1 Protein and amino acids composition

Mesopelagic fish is a good protein source and is comparable to herring and blue whiting (Table 1). The essential amino acid composition of both *B. glaciale* and *M. muelleri* were well balanced and similar to blue whiting. *B. glaciale* has higher levels of certain amino acids than *M. muelleri*, particularly valine, leucine, phenylalanine, methionine and lysine (Table 11). Compared to requirement for essential amino acids, both mesopelagic species have an amino

acid pattern that cover the requirement for essential amino acids reported for Atlantic salmon (Table 11).

Table 11 Amino acid composition (% of dietary protein) and amino acids requirements for Atlantic salmon (Salmo salar L)

	<i>M. muelleri</i> *	<i>B. glaciale</i> *	<i>M. poutassou</i> **	Amino acids requirements for Atlantic salmon (% of CP)***
Arg	5.10±0.24	5.70±0.29	5.24	4.80
His	1.90±0.11	2.10±0.14	1.75	
Iso	3.40±0.20	3.80±0.16	4.17	
Leu	6.40±0.37	7.30±0.32	7.32	
Lys	7.00±0.44	8.20±0.36	8.38	5.00
Met	2.50±0.06	3.10±0.30	2.77	1.73
Phe	3.70±0.18	4.10±0.18	3.67	2.66
Thr	3.70±0.23	4.00±0.19	3.95	2.60
Try	1.00±0.07	1.00±0.04	0.98	
Val	4.20±0.32	5.90±0.29	4.69	

*Olsen et al., 2019; ** www.sjomatdata.hi.no; *** NRC (2011)

4.3.2 Content of lipid and fatty acids

M. muelleri and *B. glaciale* has several fat stores in the body, especially around the digestive tract, the gonads, the liver, by the light organs, under the fish skin and intramuscularly (Falk-Petersen *et al.*, 1986). These lipid stores are used for both energy storage and neutral buoyancy and explain high lipid content in the mesopelagic fish species in the Norwegian sea (Falk-Petersen *et al.*, 1986). The lipid content of dry matter vary between 35- 47% for *B. glaciale* and 40–55% for *M. muelleri*. The smaller sizes, fish below 30 mm in both species, have lower lipid content, ranging 25–30% of dry mass (Skarpeid, 2019, Olsen *et al.*, 2019, Wiech *et al.*, 2020, Nordhagen *et al.*, 2020).

A significant difference between *M. muelleri* and *B. glaciale* is that the depot lipid is stored as triacylglycerols in the former and as wax esters in the latter (Nordhagen *et al.*, 2020). Only trace amounts of wax esters is observed in *M. muelleri* (Falk-Petersen *et al.*, 1986, Skarpeid, 2019, Wiech *et al.*, 2020). The content of wax lipids in *B. glaciale* has been reported to be 82%-90% of the total lipid content (Wiech *et al.*, 2020, Nordhagen *et al.*, 2020). Wax esters are more

hydrophobic than three acyl glycerol (TAG) and poorly digested in most mammals, including humans. Consequently, high intake may cause digestive disturbances such as stomach cramps, steatorrhea (absorption problems) and keriorrhoea (oily diarrhoea). Mesopelagic fish is not harvested and used for humans consumption (Catul *et al.*, 2001; IMR, 2017). Unlike humans, salmonids can digest and absorb wax esters, although the digestion of wax esters are slower than TAG. However, a medium amount of wax esters (30% of the lipid) in the feeds are proved to be well utilized by the Atlantic salmon (*Salmo salar*) (Olsen *et al.*, 2014)

High amounts of monounsaturated fatty acids were found in *M. muelleri* and *B. glaciale*, mainly 18:1n-9, 20:1 n-9, 20:1n-11 (gadoleic acid) and 22:1n-1 (cetoleic acid), followed by SFA and the lowest proportion of PUFA (Falk-Petersen *et al.*, 1986, Skarpeid, 2018, Alvheim *et al.*, 2020). It is assumed that cetoleic acid, which is especially high in *B. glaciale* species, stimulates the capacity of Atlantic salmon cells to produce EPA and DHA, and improve the retention of EPA and DHA (Ruyter *et al.*, 2016). Although the amount of PUFA is relatively low, the levels of 20:5n-3 (EPA) and 22:6n-3 (DHA) in the lipids can still be compared to those in surface pelagic fishes, like mackerel, sardine (Baby *et al.*, 2014, Koizumi *et al.*, 2014) and blue whiting (Table 12). The levels of EPA and DHA contents found in *M. muelleri* and *B. glaciale*, on average, 1.03 and 1.0 g 100g⁻¹ lipid, and 2.22 and 2.24 g 100g⁻¹ lipid, respectively. In general, *B. glaciale* had higher levels of EPA and DHA when compared to *M. muelleri*, although they were also characterised by having a much higher variation in total n-3 LC-PUFA content, regardless of fish size (Olsen *et al.*, 2019)

Table 12 Absolute and relative values of selected fatty acids in 2 mesopelagic fish from three fjords in western Norway, and for comparison Blue whiting (*Micromesistius poutassou*) (mean ± SD) (Alvheim *et al.*, 2020)

	<i>B. glaciale</i> g/100 g ⁻¹ ww	<i>M. muelleri</i> g/100 g ⁻¹ ww	<i>M. poutassou</i> g/100 g ⁻¹ ww*
Sum SFA	0.90±0.21	3.78±2.04	0.74
18:1n-9	1.35±0.43	1.35±0.79	0.44
20:1n-9	0.53±0.17	1.52±0.89	0.21
Sum MUFA	4.03±1.14	7.82±4.41	1.25
Sum n-6	0.21±0.05	0.31±0.15	0.10
20:5n-3	0.41±0.10	0.61±0.35	0.23
22:6n-3	0.68±0.13	1.11±0.57	0.53
Sum n-3	1.54±0.35	2.44±1.31	0.92
Sum PUFA	1.85±0.41	2.89±1.52	-

* www.sjomatdata.hi.no

Compared to commercially available fish oils, the mesopelagic species contained higher amounts of DHA compared to EPA. DHA is assumed to be critical as a structural component to PL, while EPA has been recognized to be an essential bioactive FA (Bou *et al.*, 2017). It is demonstrated that while DHA alone can maintain a healthy fish, EPA in a diet cause a range of physiological and biochemical benefits beyond those achieved by inclusion of DHA alone (Glencross *et al.*, 2017). The difference of DHA level compared to EPA in the mesopelagic fish is however insignificant, the ratio is suggested to be 0.05 (Alvheim *et al.*, 2020).

4.3.3 Vitamins, minerals and undesirable substances

Gopakumar *et al.* (1983) reported that lanternfishes are a good source of potassium, sodium and calcium. Most of the values observed by Olsen are in the same range as those reported for herring, capelin and anchovy meals, with exception for phosphorus which was lower than regular fish meal (Olsen *et al.*, 2019). Both species *B. glaciale* and *M. muelleri* are also proved to contain high levels of vitamin A1 (retinol) (Olsen *et al.*, 2019). Concentrations of heavy metals and organic pollutants are generally low in *B. glaciale* and *M. muelleri* in the Norwegian sea and are shown to be far below the maximum levels given in the feed regulation (Wiech M *et al.*, 2020)

4.4 Harvesting and regulations.

In the last 50 years, a number of attempts to commercialize mesopelagic fisheries for the production of fishmeal have been carried out (IMR, 2017). However, establishing economic fishing has been unsuccessful for a long time, mainly due to lack of research and investment in technology development (IMR, 2017). Following a new global estimate of this resource (Irigoiien *et al.* 2014) and an increasing need for marine raw material for fish feed, a new attempt to develop a fishery for mesopelagic species was initiated in Norway in 2015. The Institute of Marine Research launched the "Mesopelagic Initiative" in 2016 and significant resources have been invested in research and experimental fishing. Research on mesopelagic ecosystems has also been included in the EAF-Nansen Programme's Science Plan as part of a dedicated theme, which aim is to understand the biology, diversity, and ecological role of mesopelagic fish and jellyfish (FAO).

Globally, only a few attempts have been made to develop commercial mesopelagic fisheries in the Gulf of Oman and in the southern parts of Iceland. Otherwise it is still in the experimental stage. Over 5000 tonnes of light-spotted fish (*Lampanyctodes hectoris*) were landed during an experimental harvesting in South Africa in 2018 (Bjørndal *et al.*, 2020). An experimental cruise in Norway during two three-weeks periods in summer 2019 gave significant results (around 1500 tonnes, salmon herring and krill) which was delivered for fish meal and oil production. The catch size was relatively small, but considering that this is a fishery under development and a very short harvesting period, the catch result must be characterized as promising (Bjørndal *et al.*, 2020).

The Directorate of Fisheries has issued a number of experimental permissions for fishing of mesopelagic species in Norway (Directorate of Fisheries, 2018). Two long-term permits have been issued to two fishing companies for the period 2017-2021. The permits apply in Norway's economic zone north of 62 ° N outside 12 nautical miles from the baselines at bottom depths of 1000 meters or deeper and in international waters in the Irminger Sea. In addition, four shipping companies were granted permissions for harvesting south of 62N in the North Sea and four one-year permissions for harvesting north of 62N in 2019.

While most commercial fish stocks in the north Atlantic are regulated with total allowable catch (TAC) and individual vessel quotas, neither TAC's nor rules for bycatch are yet implemented for harvesting mesopelagic fish in Norway.

4.5 Challenges.

4.5.1 Ecological challenges

Mesopelagic community is a key resource for higher trophic levels, being a prey for key fisheries stocks and marine mammals as well as it plays an essential role in carbon export and thus climate regulation (Hudson *et al.*, 2014, St John *et al.*, 2016). Overexploitation of this community can potentially result in trade-offs related to climate regulation and conservation of biodiversity. It is therefore crucial to ensure good knowledge of the biodiversity in the mesopelagic community and the role that the various species play in the marine ecosystems, before a large-scale commercial harvesting can start.

4.5.2 Technological limitations.

Mesopelagic fish have at times proved difficult to catch, so the capture technology must be developed to harvest such organisms efficiently (Bjørndal *et al.*, 2020). The experimental cruise, carried out in the Norwegian waters in 2019, showed that the vessels without sorting grind in their trawl, had the best catch size (Bjørndal *et al.*, 2020). Absence of a sorting grind results in significant amounts of quota-regulated species as by-catch. Consequently, mesopelagic fishery may contribute to higher fishing mortality for quota-regulated species, such as herring, mackerel, blue whiting and seith.

Mesopelagic zone is inhabited by diverse taxa such as myctophid and stomiiform fish, pelagic small shrimps, squids and various groups of gelatinous zooplankton (Garsia- Seoane *et al.*, 2020). Harvesting of intended species is difficult with the existing technology. Future harvesting of mesopelagic fish will be a mixed species requiring knowledge of both the community composition and the chemical composition. It is further suggested to develop a combined real-time optical and/or multifrequency acoustic system that will help to improve species identification or/and a selective trawl that target only fish and release unwanted species (Standal *et al.*, 2020)

The profitability of a future mesopelagic fishery depends on the type and size of the pelagic trawler, the distance to the fishing areas, the price of fish and the type of strategy (full-time mesopelagic fishery of combined fishery with other commercial pelagic species) (Standal *et al.*, 2020). It is yet unclear whether today's deep-sea pelagic vessels are suitable- or large enough to develop a sufficient large-scale mesopelagic fishery. Anyway, taking into consideration the current technology and its operating costs, a future commercial mesopelagic fishery is only possible if the demand and, hence, the price for mesopelagic catches increase (Prellezo *et al.*, 2019).

4.5.3 Nutritional limitations.

Mesopelagic fish are small fatty fish with thin skin and is readily spoiling after harvesting (Huss *et al.*, 1995). They are fragile and disintegrate quickly. The fish skin dissolves when the fish is hauled on board, and the physical damage result in easy access for enzymes and spoilage bacteria. Enzymatic and bacteriological changes cause rapid quality deterioration. Moreover, the large amount of polyunsaturated fatty acid in fish lipids makes them highly susceptible to

oxidation, resulting in the development of off-flavors, and loss of nutritional quality. Harvesting technology, handling and storage on board should address these challenges.

High levels of wax esters in *B glacialis*, high levels of cadmium, arsenic and fluoride in krill and shrimp, that are predominating species in the mixed mesopelagic layers, as well as high levels of chitin, may limit the use of the mesopelagic species as a feed ingredient.

5. Discussion.

The Norwegian government's ambition is that Norway by 2050 will become the world's leading seafood nation, producing 5 million tons of sustainable aquaculture products (Parliament report no. 22 (2012-2013)). This is almost four times the amount of the current seafood production. Two main challenges that need to be resolved to reach such ambitious goal, are salmon lice and demand for feed (PwC seafood barometer 2017). Increased production will demand more high quality aqua feeds. The world's fish resources are already fully exploited or overexploited, so the probability of increasing the supply of fish meal and fish oil from reduction fisheries is minimal. In order to relieve the pressure on wild fish resources, most of the fish meal and oil in the aquafeeds have been substituted by plant ingredients. Plant-based ingredients as alternatives for marine ingredients have some limitations, such as unbalanced amino acid profile (Berge *et al.*, 1998, Breck *et al.*, 2005), poor digestibility (Green *et al.*, 2013, Hartviksen, 2014, Booman *et al.*, 2018), lack of n-3 long chain polyunsaturated fatty acids (Bell *et al.*, 2002, Berge *et al.*, 2009, Menoyo *et al.*, 2010, Bou *et al.*, 2017), presence of anti-nutritional factors (Krogdahl *et al.*, 2010), and high fiber content (Arnesen *et al.*, 1995, Hemre *et al.*, 1996, Polakof *et al.*, 2012, Kamalam *et al.*, 2017). Moreover, significant environmental and socio-economic effects of increased use of plant-based ingredients have emerged (Boissy *et al.*, 2011, Pahlow *et al.*, 2015). Demand for novel alternative sources of key ingredients for aquafeeds is urgent.

Fish meal and fish oil of good quality are ingredients with superb nutritional quality for fish. The protein in fish meal is highly digestible and contains all essential amino acids in a favorable composition. Fish oil is particularly rich in the n-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA), especially EPA and DHA. Alternative ingredients with similar nutrient composition as in fish meal and oil are therefore preferable. The long chain n-3 fatty acids are mainly obtained from marine environment, and it may therefore be logical to accentuate the search for alternative sources there.

The novel marine ingredients addressed in this thesis, have favorable nutritional profile, and generally all the nutrients to satisfy the nutritional requirements of Atlantic salmon. Fish meal produced from by-products have similar nutritional profile to fish meal produced from reduction fisheries. However, high ash content in by-products is not necessarily positive because it reduces the energy density of the feed for farmed fish. *C. finmarchicus* have a high protein content (44-52% DM) and a well-balanced amino acid profile. Mesopelagic fish species

have lower protein content (35-41% DM), but it is still comparable to the protein content of herring and blue whiting (Alvheim *et al.*, 2020). The composition of essential amino acid in the mesopelagic fish are well balanced (Olsen *et al.*, 2014) and the content can very well cover the requirement of Atlantic salmon. One limitation for use of mesopelagic fish species may be that they also contain several contaminants and substances that potentially can cause negative health effect, like heavy metals and organic pollutants (Olsen *et al.*, 2019, Wielch *et al.*, 2020). Although the levels are below the allowed limits, it is anyway a concern that give negative perception by the customers.

The lipid content of fisheries by-products, *C. finmarchicus* and mesopelagic fish species is superior to plant-based ingredients and comparable to fish oil from reduction fisheries. Between 54 and 69% of lipids in cod, saithe and haddock are stored in liver, so if liver is sorted out, the rest of by-products from white fish fisheries will generate a product with low amounts of lipids (Falch *et al.*, 2006a). The amount of liver available from the Norwegian fisheries is however limited. Calculating that liver makes 4-6 % of round weight in cod, haddock and saithe (Falch *et al.*, 2006b), no more than 40 thousand tons liver, or 25 thousand tons lipid were available from the Norwegian white fish fisheries in 2019. Almost 90% of lipids stored in the liver are triacylglycerols, which is similar to fish oil produced from whole fish. This fact makes liver more suitable in aquafeeds than *C. finmarchicus* and *B. glaciale*. Although *C. finmarchicus* is proved to be an excellent source of lipids, (60% lipids of its total dry weight), most of the lipids reserves (80-90%) are stored in form of wax esters (Kattner *et al.*, 1987, Smith, 1990, Jónasdóttir, 1999, Scott *et al.*, 2000, Lee *et al.*, 2006). The content of wax esters in *B. glaciale* is reported to be 82%-90% of the total lipid content (Wiech *et al.*, 2020, Nordhagen *et al.*, 2020). Wax esters are poorly digested in mammals and humans, and cause digestive disturbances such as stomach cramps, steatorrhea (absorption problems) and keriorrhoea (oily diarrhoea). Atlantic salmon is proved to be able to digest and absorb wax esters, there is however an upper recommended level of 30% of the dietary lipids in feed for Atlantic salmon, that the fish is able to utilize without negative consequences (Olsen *et al.*, 2004, Bøgevik, 2011). All the three studied ingredient contain EPA and DHA, comparable to fish oil and are thus superior to plant-based ingredients. The quality that makes *C. finmarchicus* a particularly interesting resource in salmon feed is high content of carotenoid pigment, that makes Calanus oil an excellent natural alternative to synthetic astaxanthin.

Based on protein and lipid content, amino acid pattern and fatty acid composition, all the three studied ingredients seem to have a good potential as a feed ingredient. There are however a number of challenges, associated with each of them. Although the Norwegian government has recently issued licenses for commercial harvesting of *C. finmarchicus*, the participation has been limited. According to the data from the Directory of Fisheries, 350 tons *C. finmarchicus* were landed in 2019 and none so far in 2020 (per 01.10.20). Complex harvesting technology and limited production capacity are mentioned as a significant limiting factor. New technological solutions for harvesting *C. finmarchicus*, are being actively tested (Ole-Petter Pedersen, Calanus AS- pers. com). Commercial technologies for efficient harvesting is still not available. Specific solution for targeted harvesting of mesopelagic fish has been proposed, but not yet implemented (Grimaldo *et al.*, 2020). Increased utilization of byproducts has been prioritized by Norwegian government from a resource utilization perspective, and most of the available by-products are already being exploited (SINTEF, 2020). There is still a potential to increase the utilization, especially from the whitefish sector. On board vessels, space is a limitation and often the by-products is thrown overboard. Although larger vessels seem to have better premise for storage and processing of by-products, the ocean going vessels produce least by-products in Norway (SINTEF, 2020). Fishermen should be either encouraged to collect and make use of by-products with some economic benefits, or obligated to do so by regulatory requirements.

According to the report from SINTEF, 1 371 322 tones feed were used in Atlantic salmon aquaculture in Norway in 2017. The feed contains many different ingredients (Figure 9), the major parts are crop-based proteins (40%), where soy protein is the main ingredient (29.55%), and crop-based oil (20%), which is a 100% rapeseed oil.

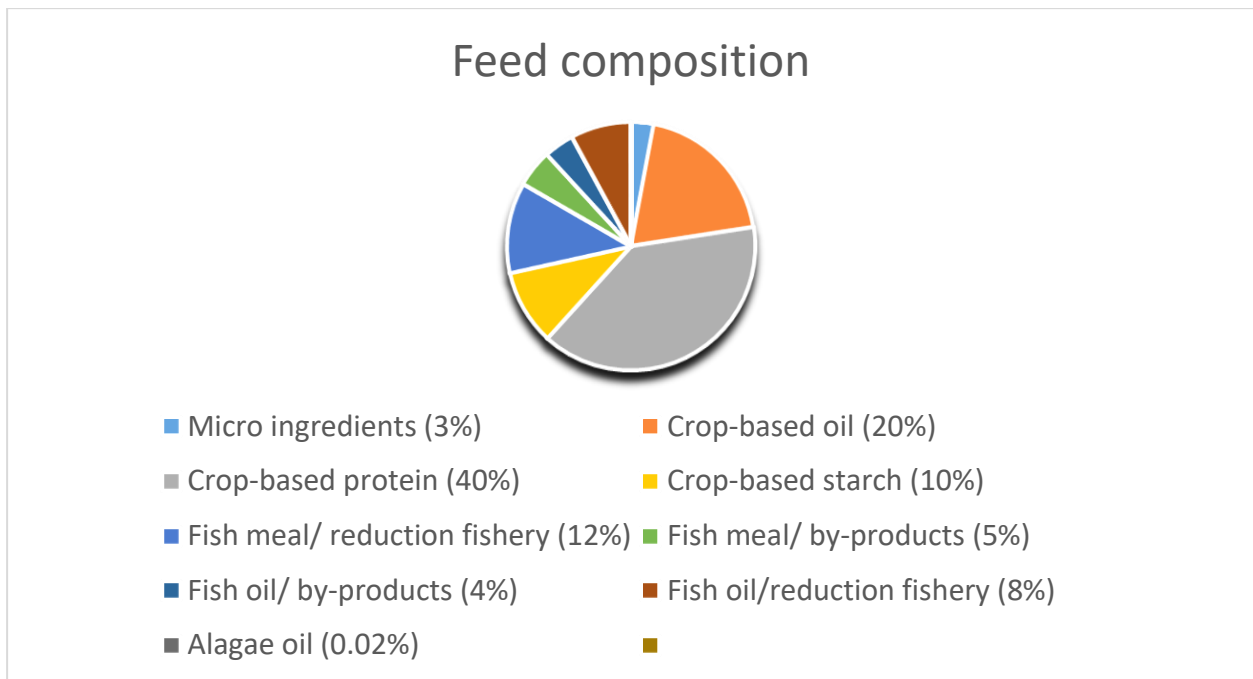


Figure 10 Feed ingredients used for fish feed in 2017 (data from three major feed producers) (SINTEF, 2019)

The mesopelagic biomass is estimated to be 10 000 million tons in the world ocean (Irigoiien *et al.*, 2014). There is no specific number for biomass in the Norwegian sea, it is however assumed to be considerable. Roughly 2,1 million tons of mesopelagic fish (with an average protein content of 13%) is needed to fully replace the use of soy protein in salmon feed in Norwegian aquaculture in 2017. Additional 1,2 million tons of mesopelagic fish are needed to replace fish meal. The enormous biomass of mesopelagic fish makes this resource a good alternative in aquafeeds.

The biomass of *C. finmarchicus* in the Nordic seas (Norwegian, Icelandic and Greenland seas) is estimated to 70-80 million tones (Melle *et al.*, 2014). A Norwegian quota for commercial fisheries is 254 000 tons annually. Based on the size of quota and the fact that *C. finmarchicus* consist of 10-11% protein and 5-8% lipids, a commercial harvesting can theoretically contribute with 25 000 tons proteins and over 12 000 tons lipids. The commercial feeds for farmed Atlantic salmon, consisted of 300 tons of crop-based protein, 164 000 tons of fish meal from reduction fisheries. Almost 275 thousand tons of crop-based rapeseed oil and 104 000 tones fish oil from reduction fisheries represented the lipid content. It is highly unlikely that protein and oil from *C. finmarchicus* will make a big contribution as feed ingredient in salmon taking into account the low quota, the current harvesting technology and cost of obtaining oil from zooplankton.

The unique nutritional composition of *C. finmarchicus* makes it however a valuable niche product, for example, as ingredient in starter feed, an astaxanthin-rich protein meal and oil component or a feeding attractant (Calanus AS).

According to SINTEF (2020) almost 100% of by-products for pelagic fisheries and 61% from gadiform fisheries are already being utilized, so there is a relatively small amount of unutilized volume left. Around 2,5 million ton fish was discharged by Norwegian vessels in 2019, of those over 700 thousand tons of gadiform species (SSB, 2020). The white fish fisheries generated around 320 000 tones by-products, while 181 000 tons of those were utilized (SINTEF, 2020). Approximately 57% of by-products were used in aquaculture feed for salmon and other marine species in 2018. If there were 100% utilization of by-products from white fish fisheries, this would have generated 75 thousand tons extra ingredients for aqua feeds. Assuming 65% protein content in by-products from gadiform species, 49 thousand tons of protein can potentially be used in salmon feed. This constitutes 17% of the imported soy protein (281 000 tons) and 30% of the fish meal protein from reduction fisheries (approximately 164 000 tons) used in salmon feed in Norway in 2017 (SINTEF, 2019). Based on available volumes of by-products from the Norwegian fisheries, especially in case of products from the whitefish fisheries, it is hardly realistic to consider by-products as a potential replacement to fish meal and fish oil and plant-based ingredients in Norwegian aquafeeds. A maximum utilization of all by-products should however be argued for, in order to contribute to ecologically and economically sustainable development and increase activity in the coastal districts.

The production cost for one kilo Atlantic salmon has been constantly increasing since 2012. The main share of the production cost increase was feed costs (PwC seafood barometer 2017). The Covid-19 pandemic has further aggravated the problem of expensive imported aquafeed. Economic viability is an important sustainability element and should be systematically taken into account. Norwegian ingredients may not be competitive to imported sources, if the price is significantly higher, unless the use of alternative ingredients have economic or regulatory incentives, that encourage substitution away from fishmeal and fish oil and plant-based ingredients.

In order to produce 5 million tons of aquaculture products in the future, which is 4 times more than today, the Norwegian producers will have to increase the amount of feeds accordingly or optimize the feed formulations to achieve the most efficient feed conversion ratios (FCRs). Either way, the growth should be sustainable. All Norwegian fisheries are a subject of well-

planned management. Regulation and control regime reduces the risk for overexploitation of wild fish stocks. Utilizing all parts of the fish, also by-products, is warranted both from an ethical and ecological point of view. Perceived environmental sustainability of the fishing industry, economic and social benefits from creating processing jobs, will ultimately contribute to the long-term sustainability and public accept for production of aquafeeds and aquaculture in general. Utilization of *C. finmarchicus* is far more controversial by society, than fish by-products. Although stock assessment were well documented prior to issuing the commercial concessions, harvesting of *C. finmarchicus* was disputed. Despite sustainable management, low harvesting and production volumes, the public opinion is negative to the harvesting of *C. finmarchicus*. Negative perception of harvesting organisms low in the trophic food web in general, as well as harvesting methods and areas in particular, may eventually restrict the possibility for utilization of *C. finmarchicus* as ingredient for aquafeeds. Mesopelagic fish remain one of the least investigated biomasses in terms of distribution, abundance, fishing methods and product development. Unsustainable utilization can have negative effects, by reducing biodiversity and by compromising the oceans' role in climate regulation. Comprehensive investigations are decisive for establishing an extensive knowledge. Public attitude towards potential harvesting of these resources is difficult to predict. The notion of acceptability is complex and depends on among other cultural and social factors (IUCN). Importance of communication, to make the knowledge about novel ingredients more coherent and acceptable, should not be underestimated.

Understanding the environmental impact of aquafeeds is important for assessing and improving the environmental performance of aquaculture (Paratryphon *et al.*, 2004). Tools such as Life Cycle Assessment (LCA) are used to study environmental impacts of feed ingredients. Life cycle assessment is a methodological framework to estimate the environmental impacts attributed to the life cycle of a product (ISO, 2006). A number of studies are carried out on different ingredients, using the LCA (Samuel- Fitwi *et al.*, 2013, Silva *et al.*, 2018, Maiolo *et al.*, 2020). Sustainability is usually evaluated in three dimensions, economic, environmental and social, and all of them are eventually essential for sustainable production. Sourcing new ingredients for aquafeed require thorough assessment of many dimensions including nutritional quality as well as sustainability. The Norwegian seafood federation, Norway's largest association for seafood industry, has in its vision "Seafood 2030 - a blue change of pace" committed to sustainable production and consumption for Norwegian seafood industry, closely linked to the UN's sustainable development goals. The federation suggests that environmental

impact can be reduced by replacing plant-based ingredients in aquafeeds with mainly marine ingredients by 2030. The main requirements for future development, is that all marine resources, including “new” ones, must be managed sustainably. Research and development, financed and carried out by both the community and the industry itself, will be an important prerequisite for success (Sjømat Norge, 2017). “Innovation should be measured in decades, not years,” said Ocean Harvest Technology’s CEO Graham Eliis during the Global outlook for aquaculture leadership conference panel in October 2020. It is therefore even more important not to haste with implementing novel resources until the knowledge is profound.

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