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Can mesopelagic mixed layers be used as feed sources for salmon aquaculture?

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ABSTRACT

Salmon aquaculture is in great need of good quality balanced protein and lipid sources, particularly marine omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA), to sustain a further development of the industry. One possibility is to harvest mesopelagic marine layers. Therefore, the current project analysed mesopelagic hauls from three cruises (November 2015 to October 2016) collected from the inner fjord systems around Bergen and in open-waters off Tromsø and Ålesund, Norway. Jellyfish, krill, shrimps and small amounts of the mesopelagic fish, *Maurolicus muelleri* and *Benthosema glaciale*, dominated the mixed mesopelagic hauls. Lipid content ranged between 35-40% of dry matter with two samples from autumn being 21 and 13%, with the latter haul being almost exclusively krill. In contrast, *M. muelleri* and *B. glaciale* had lipid contents of around 54 and 47% respectively. Overall, lipid was a relatively good source of marine n-3 LC-PUFA, EPA and DHA, being in the range of 15–20% of fatty acids which increased in lean samples. However, many of the trawl hauls contained wax esters (7 out of 9 hauls), equivalent to 40% or more of the lipid, with *B. glaciale* containing almost 90% wax esters of lipid. This presents a challenge if used in salmon diets, as their utilisation is limited. Protein contents ranged between 45-50%, increasing in lean samples. The essential amino acid content was well above the requirements for Atlantic salmon (*Salmo salar*) with *B. glaciale* generally containing higher levels compared to *M. muelleri*. Leucine, lysine and valine levels were particularly high. Hauls from open-waters contained mixtures of amphipods resulting in cadmium levels exceeding the maximum allowable level in feedstuffs. Arsenic levels were high or borderline. Reducing crustacean mix in hauls appear to be the only option to reduce these levels, whereas mesopelagic fish contained low levels of all heavy metals. In summary, the mesopelagic layer contains protein and lipid sources that could supply raw materials to the salmon aquaculture industry. However, high levels of wax esters, cadmium and arsenic needs to be addressed.

1. Introduction

The continual growth of the global population coupled with its demand for seafood has placed further pressures on the wild capture fisheries that are already at or beyond their capacity (FAO, 2018). Aquaculture, the farming of seafood, has sought to resolve this issue and now supplies over 50% of the seafood destined for the table market (FAO, 2018). However, many of the farmed species, particularly carnivorous marine species such as Atlantic salmon (*Salmo salar*), seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*), have traditionally relied upon the inclusion of raw ingredients sourced from wild fisheries in order to satisfy their nutritional requirements. Efforts to replace these finite ingredients with alternatives, typically of terrestrial origin, have resulted in the Norwegian salmon feed industry shifting from 90% marine ingredient inclusion in feeds in 1990 to less than 30% inclusion in 2013 (Ytrestøyl et al., 2015). While many of the issues surrounding the substitution of the marine protein content (fish meal) with plant-based meals have largely been addressed, e.g. amino acid

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deficiencies through supplementation (Gatlin et al., 2007), many anti-nutritional factors are still present in extensively processed plant proteins that can still cause nutrient malabsorption and inflammatory conditions (Zhou et al., 2018). Moreover, the decrease in fish oil use, with a concomitant rise in plant oil inclusion, has been of particular concern owing to the associated decline in the health-beneficial omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexanoic acid (DHA; 22:6n-3) in the farmed fish product (Sprague et al., 2016). The level of EPA and DHA in feeds have been reduced from 20% of the dietary lipid in the late 1990's to less than 5% in current feeds (Einen et al., 1998; Ytrestøyl et al., 2015), subsequently compromising the value of farmed salmon as a prime source of these fatty acids for human consumers (De Roos et al., 2017; Sprague et al., 2016). There is also increasing concern that land areas that can be used for production of food for direct human consumption are being channelled into production of feeds for salmon. However, few other sources can supply raw materials in the quantities needed for a growing salmon industry without seriously affecting the production cost.

Based on the above considerations, there is still a great interest in finding alternative marine-based resources that could be suitable for providing protein and lipid for salmon feeds. However, in the ocean, pelagic fisheries are exploited to the maximum level with no further option for increase. Nevertheless, at lower trophic levels, there are large biomasses of organisms with a high nutritional value. Both krill and copepods have been explored in the past and have shown promising results (Olsen et al., 2010; Suontama et al., 2007a). In recent years the mesopelagic layer, including mesopelagic fish, has received more attention. Mesopelagic fish occupy the intermediate layers between 100 to 1000 m depth, often migrating up to higher levels during night and down again during daytime. The global biomass of mesopelagic fish is enormous and was estimated in the 1980's at around 1 billion metric tons (MT) (Gjøsæter and Kawaguchi, 1980), although a more recent study estimates this to be higher, between 6 to 10 billion MT (Irigoien et al., 2014).

In the coastal areas of Norway the mixed mesopelagic layers have been shown to contain varying contents of mesopelagic fish like *Maurolicus muelleri* and *Benthosema glaciale*, as well as krill, jellyfish and shrimps (Dalpadado et al., 1998; Skjoldal et al., 2004). Thus, any potential commercial fishery would be expected to include these mixed layers as a whole based upon the species present at the time of harvest. Consequently, the catch composition would be expected to vary in terms of its nutrient composition and suitability as ingredients for salmon feed. Furthermore, these variations may also affect ecosystem recruitment which may need to be monitored. The present study therefore evaluated the species content and nutritive value of mixed mesopelagic layers caught off the Norwegian coast as potential ingredients for aquaculture feeds. To this end, harvests were analysed for their lipid, protein, amino acid, fatty acid (and alcohol) and mineral and heavy metal levels. In addition, the predominating mesopelagic fish species, *M. muelleri* and *B. glaciale*, were separated out in some of the hauls and analysed for nutrient compositions.

2. Materials and Methods

2.1. Sample collection and preparation

Samples were collected by trawl hauls on three separate transect cruises between November 2015 to October 2016 using the Norwegian marine research vessel G.O. Sars, operated by the Institute of Marine Research and the University of Bergen (Bergen, Norway). The first cruise (Fjord15) was conducted between the 15 to 21 November 2015 in the inner fjords south of Bergen (Norway); the second cruise (Fjord16) was performed in essentially the same area between 4 to June 6, 2016; and the final expedition (Ocean16) conducted between 13 to October 26, 2016 in the open waters off Tromsø and Ålesund (Fig. 1). A total of 4, 3 and 4 hauls, from the three cruises respectively, were used in analysis. Of the 11 trawls, 9 were oblique hauls that did not target any particular acoustic layer, whereas on one occasion a layer was targeted (340-330 m depth). Further details on trawl hauls depths and latitudes are given in Table 1.

The total catch of each trawl haul was weighed before larger species such as jellyfish (e.g. *Periphylla periphylla*, *Cyanea capillata*, *Aurelia aurita*) and fish species (e.g. *Pollachius virens*, *Cylopterus lumpus*, *Micromestitius poutassou*, *Scomber scombrus*, *Sebastes sp.*) were removed and weighed separately. From the remaining mixture of smaller-sized species, two separate, random subsamples were taken. One subsample was immediately frozen for the subsequent analysis of pooled nutrient composition, whereas the second subsample was weighed before sorted into species or higher taxonomic groups with each species/group catalogued and individually length-weighed. This procedure enables the back-calculation of the contribution of both large and small individual to the total catch. Subsamples of the mesopelagic fish *B. glaciale* and *M. muelleri* were sorted into size groups with small fish (0–30 mm), intermediate fish (30–50 mm), and large fish (50–70 mm). Fish over 70 mm, only *B. glaciale*, formed a separate group. All samples were immediately frozen and stored at −80 °C until analysed. Samples were lyophilised by freeze-drying, ground using a mortar and pestle until a homogenous mass was attained and stored at −80 °C until analysed.

2.2. Lipid content and fatty acid/alcohol composition

Total lipid was extracted from lyophilised samples in 20 vol of ice-cold chloroform methanol (2:1, v/v) using an ultra-turrax tissue disruptor (Fisher Scientific, Loughborough, UK) according to Folch et al. (1957). Non-lipid impurities were isolated by washing with 0.88% KCl and the lipid weight determined gravimetrically following evaporation of solvent under oxygen-free nitrogen and overnight desiccation *in vacuo* before making up to 10 mg.ml^{-1} concentrations and storing at −20 °C.

Fatty acid methyl esters (FAME) from total lipid were prepared by acid-catalysed trans methylation at 50 °C for 16 h using 2 ml of 1% (v/v) sulphuric acid (95% Aristar®, BDH Chemicals, Poole, UK) in methanol and 1 ml of toluene (Christie, 1993). FAME were then extracted and purified by thin-layer chromatography (TLC) as described previously (Tocher and Harvie, 1988), before separated and quantified by gas-liquid chromatography (GC) using a Thermo Finnigan Trace GC (Thermo Scientific, Milan, Italy) equipped with a 30 m × 0.32 mm i.d. × 0.25 μm ZB-wax column (Phenomenex, Cheshire, UK), 'on column' injection and flame ionisation detection. Hydrogen was used as carrier gas at constant pressure (175 kPa) with the oven thermal gradient from 50 °C to 150 °C at 40 °C.min^{-1}, then 195 °C at 2 °C.min^{-1}, 205 °C at 0.5 °C.min^{-1} to a final temperature of 230 °C at 40 °C.min^{-1}. Individual FAME were identified by comparison to commercial (Restek 20-FAME Marine Oil Standard; Thames Restek UK Ltd., Buckinghamshire, UK) and in-house (Marinol marine oil) standards as well as published data (Tocher and Harvie, 1988). Data were collected and processed using Chromcard for Windows (Version 2.11; Thermo Fisher Scientific Inc., Milan, Italy).

Fatty alcohol levels in samples containing wax esters were determined by methylating total lipid samples as described above for FAME and proceeding without TLC purification. GC conditions were identical to those reported for FAME analysis.

2.3. Crude protein and amino acid composition

Crude protein levels were determined by adding 5 ml of sulphuric acid (analytical reagent grade; Fisher Scientific, Loughborough, UK) together with 2 copper catalyst tablets (Fisher Scientific, Loughborough, UK) to ~0.25 g sample and digested at 400 °C for 1 h (Foss Digestor, 2040; Foss Analytical AB, Höganäs, Sweden). Total nitrogen levels were measured by Kjeldahl (Foss Kjeltec™ 2300; Foss Analytical AB, Höganäs, Sweden) and crude protein calculated as N × 6.25.

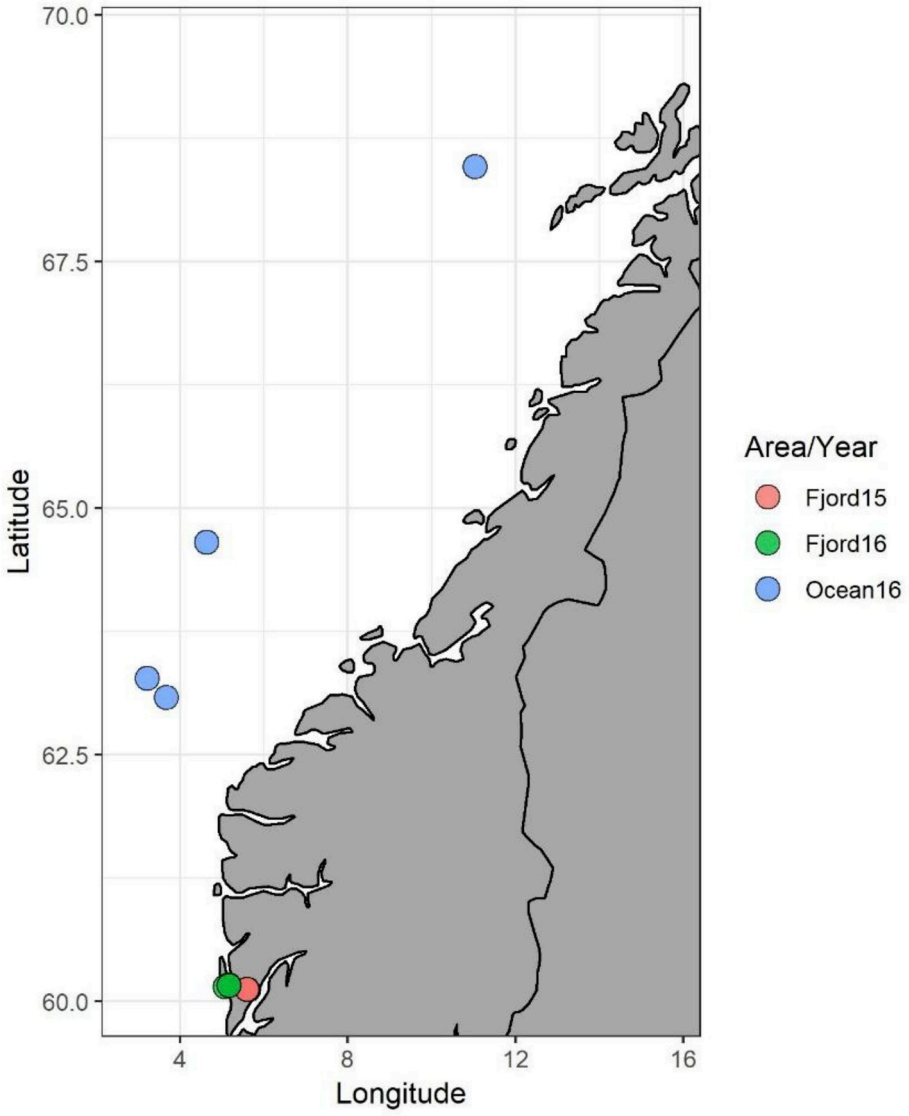

Fig. 1. Map indicating the location of the trawl hauls conducted during autumn 2015 (Fjord15, red), spring 2016 (Fjord16, green) and autumn 2016 (Ocean16, blue). Latitude on the y-axis and longitude on the x-axis explains the geographical position of the hauls. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Cruise and trawl haul information including date (YYYY-MM-DD), start and end time (UTC), latitude and longitude and the maximum and minimum depths (m) sampled. Note that no max. depth was recorded for Fjord16_188 due to a depth sensor malfunction.

Cruise	Station	Date (YYYY-MM-DD)	Time (UTC)		Latitude	Longitude	Depth (m)	
			Start	End			Max	Min
Fjord15	430	2015-11-17	12:05:00	13:00:00	60.123	5.601	430	0
	431	2015-11-17	15:13:00	15:54:00	60.120	5.597	450	0
	434	2015-11-18	20:45:00	21:51:00	60.125	5.600	407	0
	447	2015-11-20	17:36:00	18:27:00	60.123	5.590	440	0
Fjord16	186	2016-06-08	09:55:00	10:15:00	60.147	5.066	340	330
	188	2016-06-08	19:46:00	20:06:00	60.168	5.161	–	0
	191	2016-06-09	06:14:00	06:58:00	60.164	5.158	550	0
Ocean16	208	2016-10-14	16:25:00	18:03:00	68.464	11.022	1000	0
	209	2016-10-15	20:47:00	22:40:00	64.657	4.628	1030	0
	211	2016-10-20	07:46:00	09:22:00	63.081	3.659	804	0
	213	2016-10-20/21	23:47:00	00:04:00	63.276	3.208	350	0

Amino acid contents of samples were determined using the Waters ACCQ-TAG™ Ultra Method for hydrolysate amino acid analysis (Waters Corporation, Milford, Massachusetts, USA). Hydrolysis and derivatisation were performed according to manufacturer's instructions with amino acids analysed using a Waters H-Class UPLC fitted with an ACQUITY BEH Phenyl 1.7 µ UPLC column (Waters Ltd., Hertfordshire, UK).

2.4. Trace element and heavy metals analysis

Total minerals (sodium, magnesium, phosphorous, calcium, vanadium, manganese, iron, cobalt, copper, zinc and selenium) and heavy metals (lead, arsenic, cadmium and mercury) were analysed by inductively-coupled mass spectrometry (ICP-MS). Briefly, approximately 20–30 mg of sample was weighed into Teflon microwave digestion tubes before 5 ml of 69% nitric acid (Aristar® analytical grade; VWR Chemicals, Poole, UK) was added. Samples were digested in a microwave digester (MARS Xpress; CEM Microwave Technology Ltd., Buckingham, UK) in three stages consisting of 21–190 °C for 10 min at 800 W, followed by 190 °C for 20 min at 800 W, followed by a final 30 min cooling period. The digested solution was transferred into 10 ml volumetric flasks and made up to volume with deionised water. A total of 0.4 ml of this solution was transferred to 10 ml centrifuge tubes before 200 µl each of methanol and gold standard solution (Acros Organics™, 1 mg ml^{-1} in 0.5 HCl; Acros Organics, Geel, Belgium) was added for enhancing Se sensitivity and as a stabilising agent for heavy metal analysis, respectively, and subsequently made up to volume.

Samples were analysed by ICP-MS (Thermo Scientific™ iCAP™ RQ ICP-MS; Thermo Scientific, Hemel Hempstead, UK) operating in He KED (kinetic energy discrimination) mode. The ICP-MS was tuned daily before each sample run using a tuning solution (1 ppb in 2% HNO_3 and 0.5% HCl; Thermo Fisher Scientific, Bremen, Germany). Multi-element calibration curves for quantification purposes were freshly prepared using 2% (v/v) HNO_3 and diluted to appropriate concentrations (5000–15000 for phosphorus, 5005–15020 ppb for major elements and 5–20 ppb for minor elements). The internal standards, scandium and rhodium (5 ppb), were added on-line to correct for instrumental drift during the analysis. Dwell times were 0.05 s with exception for Se where dwell time was 0.5 s. A certified reference material (Fish Muscle ERM-BB42; Institute for Reference Materials and Measurements (IRMM), Geel, Belgium) was included with sample batches to assess the integrity of the sample procedure.

2.5. Quality assurance

In addition to the methodology described above, method performance is routinely assessed through the participation of interlaboratory proficiency testing including the European Federation for the Science and Technology of Lipids, orgainsed by the German Society for Fat Science (DGF, Frankfurt, Germany); Masterlab Analytical Services BV (Boxmeer, Netherlands) for analytical methods routinely used in the feed, oil and fish producing and technology sectors; and the American Oil Chemist's Society (Illinois, USA) for the fatty acid content of marine oils in which the laboratory has Approved Chemist Status.

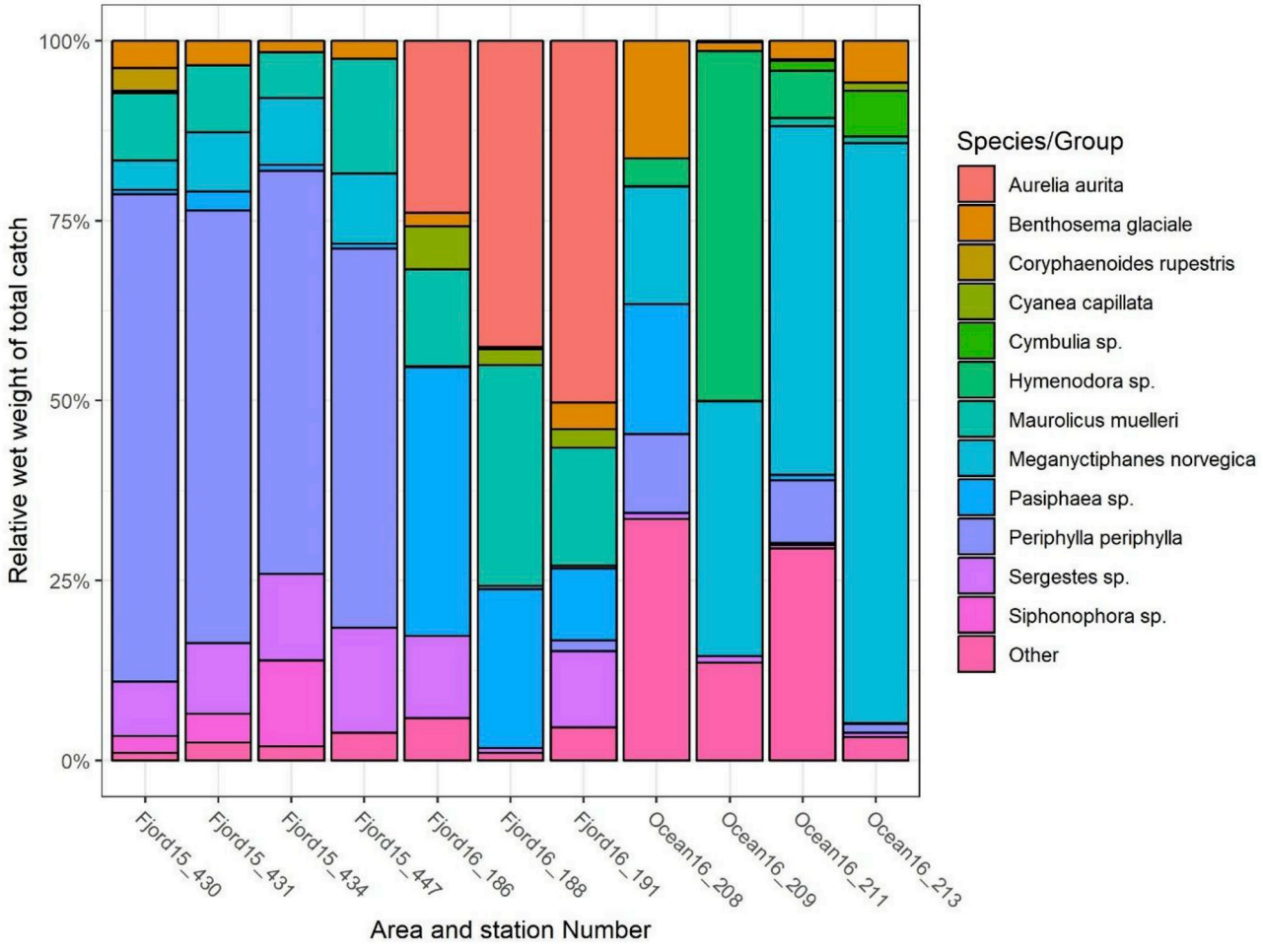

Fig. 2. Relative composition of species recorded from the wet weight from total catches. The « other » group refers to all species or groups that did not occur in any trawl haul with a density greater than 3 mg $WW.m^{-3}$.

3. Results

3.1. Trawl composition

The composition of the total catch from trawl hauls is given in Fig. 2, while Fig. 3 shows the composition of the sorted, pooled subsample, used for pooled nutrient composition. In the autumn 2015 cruise (Fjord15) in the fjord systems off Bergen, the trawl biomass was mainly dominated by the helmet jellyfish, *P. periphylla*, which always constituted more than 50% of the wet weight of the total catch (Fig. 2). After removal of *P. periphylla*, the remaining sample consisted mainly of mesopelagic fish (predominantly *M. muelleri* with smaller amounts of *B. glaciale*), pelagic shrimps (mainly *Sergestes* sp.) and krill (*Meganyctiphanes norvegica*), as well as small amounts of mysid shrimps. In addition, a quantity of siphonophores was also present, although normally in very bad condition after trawling (Fig. 3).

The spring 2016 cruise in the fjord systems off Bergen (Fjord16) revealed a different species composition compared to Fjord15. *P. periphylla* had almost disappeared, with the dominating jelly *Aurelia aurita* and to a lesser extent *Cyanea capillata*. The remaining catch consisted mainly of pelagic shrimps (*Sergestes* sp. and *Pasiphaea* sp.) as well as *M. muelleri*. Only small amounts of *B. glaciale* and krill were found to be present.

The final sampling in the autumn of 2016 (Ocean16) was carried out off the Lofoten Islands and Ålesund to the south. These hauls, not surprisingly due to the geographical distribution, varied more in composition compared to the fjord samples. The krill *M. norvegica* was a common species in all catches with one of the hauls found to consist almost exclusively this species. Of the mesopelagic fish recorded, *B. glaciale* was more common than *M. muelleri*. One trawl haul resulted in relatively large amounts of the deep-water shrimp *Hymenodora* sp. while Salps, Chaetognaths, Amphipods and Euchaeta were present in various amounts (Fig. 3).

3.2. Chemical composition

The gross chemical composition of the samples is given in Table 2. In general, there were no clear trends linking composition to season or area but, rather, composition was linked to the particular individual variations between the catches. Protein content was generally in the range 40–50% of dry matter, with total lipid being in the range 30–40%. Two hauls (186 and 213) from Ocean16 cruise showed very low lipid contents (21.1 and 13.6% respectively) and higher protein contents (53.9 and 61.6% respectively). Haul 213 was almost exclusively krill (*M. norvegica*). Ash and carbohydrate content remained relatively stable between hauls, being in the range 10–16% and 4–9% for ash and carbohydrate, respectively. The gross chemical composition of selected groups of mesopelagic fish, presented as the mean of six samples collected from hauls, is also given in Table 2. Both fish groups had comparable compositions, although *B. glaciale* displayed a trend for relatively higher levels of carbohydrates. Furthermore, lipid content of *M. muelleri* and *B. glaciale* revealed that they were relatively independent on season and area, but also highly dependent on size (Fig. 4). Small fish below 30 mm of both species had relatively lower lipid content, in the

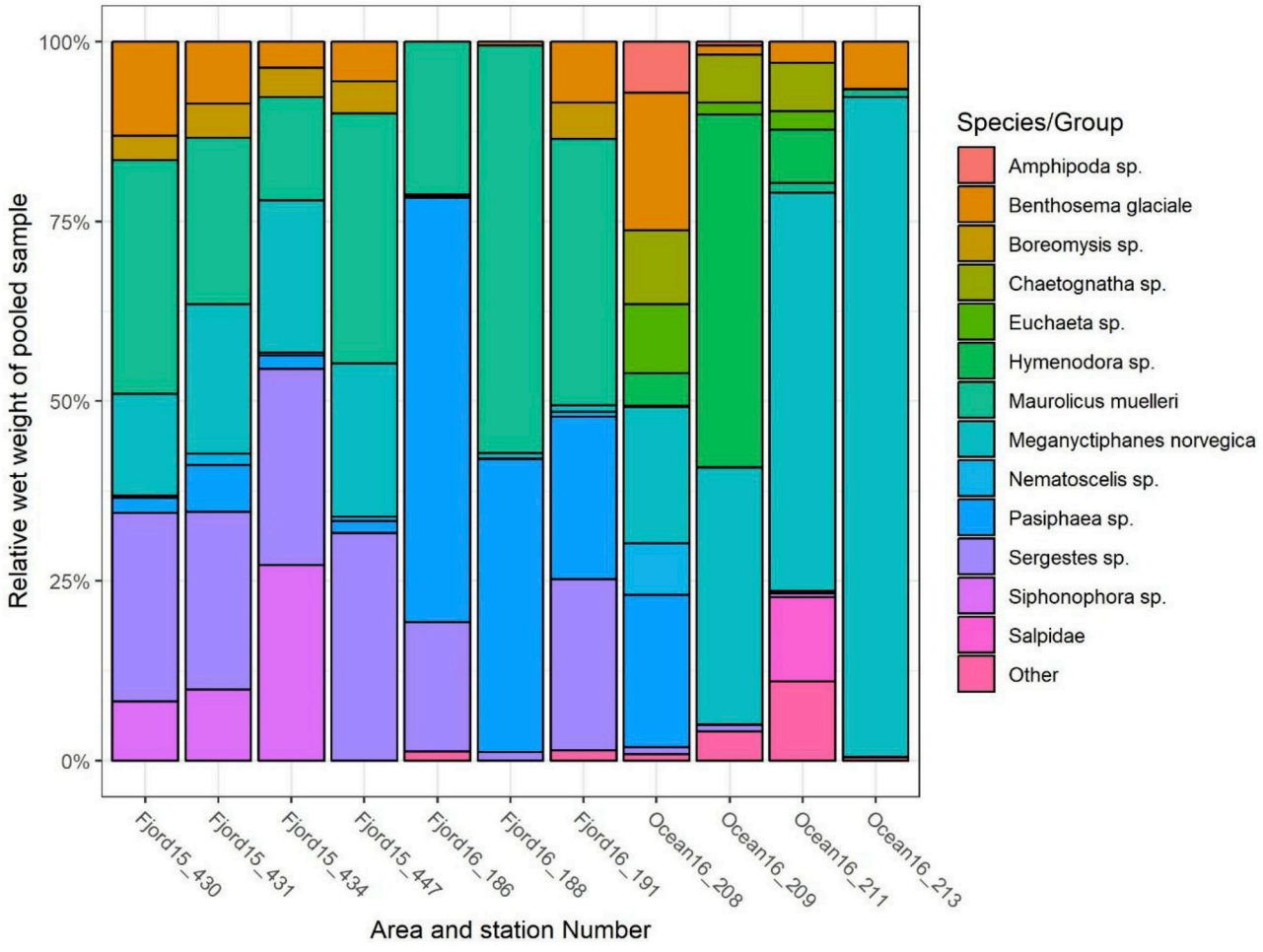

Fig. 3. Relative composition of wet weight from pooled samples used in analysis. The « other » group refers to species or groups that did not occur with a density above 1 mg WW.m^{-3}.

Table 2
Gross chemical composition (percent of dry matter) of the hauls and selected mesopelagic fish.

	Fjord15				Fjord16			Ocean16				Mesopelagic fish[a]	
Trawl station	430	431	434	447	186	188	191	208	209	211	213	*M.muelleri*	*B. glaciale*
Protein	46.0	45.1	46.7	46.0	50.6	44.0	44.2	53.9	45.6	43.2	61.5	38.9 ± 3.22	41.1 ± 1.32
Ash	11.9	11.0	11.5	11.5	12.3	9.3	12.4	16.9	12.0	11.7	15.1	7.4 ± 0.87	6.5 ± 0.40
Lipid	37.9	36.5	33.8	33.8	30.2	42.1	38.9	21.1	32.9	38.3	13.6	54.1 ± 4.83	47.5 ± 2.38
Carbohydrate	8.5	7.3	8.5	8.5	6.7	4.4	4.3	7.9	9.3	6.6	9.6	1.6 ± 1.25	4.5 ± 1.22
Energy	23.7	26.1	26.6	27.2	26.4	25.5	26.1	20.9	25.4	25.6	26.4	29.9 ± 1.22	30.6 ± 0.46

[a] Data are means of 5 samples from the different hauls.

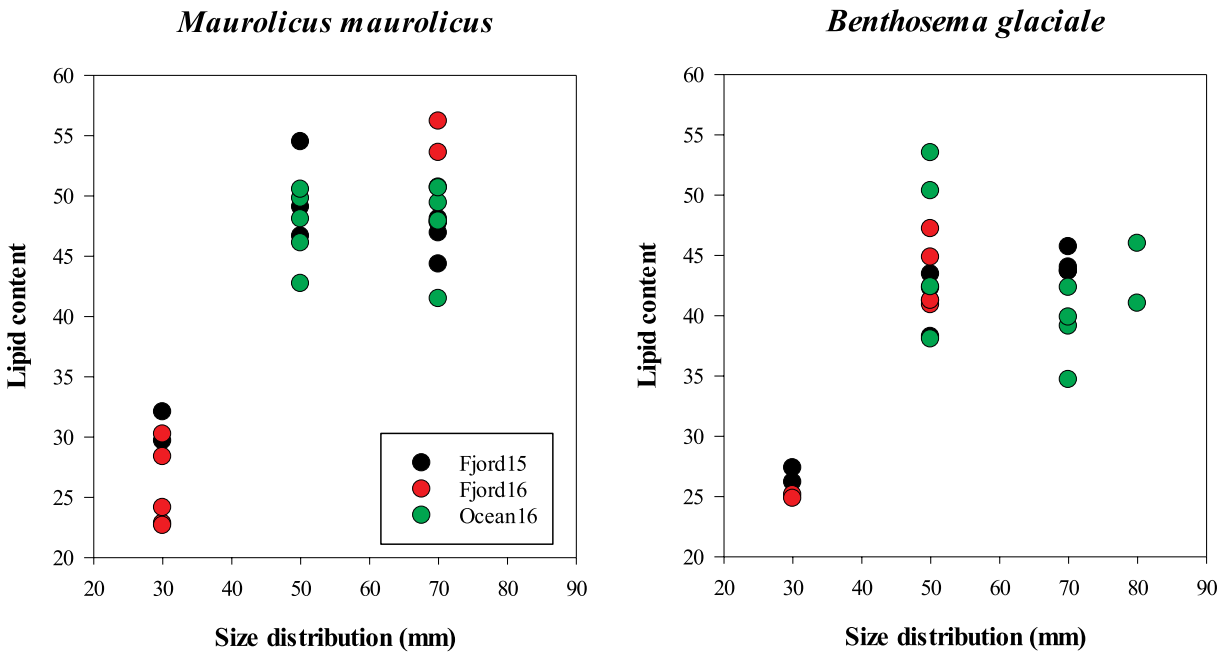

Fig. 4. Total lipid content according to size distribution of the mesopelagic fish, *Benthosema glaciale* and *Maurolicus maurolicus*. Size distribution of fish is presented in the Materials and Methods section.

range 25–30% of dry mass, whereas larger fish had high lipid contents of between 33-35% for *B. glaciale* and 40–55% for *M. muelleri*. However, the variation in lipid content was greater for *B. glaciale* compared to *M. muelleri*.

The fatty acid and fatty alcohol contents of the hauls and selected mesopelagic fish samples are given in Table 3. Samples from both autumn cruises, Fjord15 and Ocean16, contained significant amounts of wax esters as evidenced through the relatively high contents of fatty alcohols. For example, in samples from Fjord15, fatty alcohols amounted to 20–27% of combined fatty alcohols and fatty acids, which corresponds to a wax ester content of between approximately 40 to 54%. However, the Ocean16 samples varied to a greater extent, with three of the hauls showing higher levels of fatty alcohols, varying between 27-36%, which corresponded to a wax ester level accounting for 54–72% of the total lipid. In contrast, the trawl sample (213) that was almost entirely *M. norvegica* had a low content of fatty alcohols. The Fjord16 samples were very different and showed much lower levels of wax esters compared to the autumn samples. In the mesopelagic fish species, lipid of *B. glaciale* was dominated by wax esters, and showed a mean content of 43.8% fatty alcohols, suggesting that almost 90% of the total lipid content was wax esters.

The fatty acid and alcohol contents showed that most mixed hauls could provide 30–60 mg EPA and 50–100 mg DHA per gram of lipid (Table 2). This was significantly higher than the levels found in the mesopelagic fish where EPA and DHA contents for *M. muelleri* and *B. glaciale* were, on average, 10.3 and 10.0 mg.g lipid^{-1}, and 22.2 and 22.4 mg.g lipid^{-1}, respectively. For mixed haul samples, there were some differences between season and area. For example, the hauls from Fjord15 (autumn) and Fjord16 (spring) had relatively high levels of cetoleic acid (22:1n-11), while oleic acid (18:1n-9) was the predominant monoenoic fatty acid in the autumn Ocean16 hauls. The saturated fatty acid, palmitic (16:0), was relatively high in the Fjord16 samples. However, the main factor influencing the EPA and DHA contents was the total lipid content of the hauls. The relative proportions of EPA and DHA as percentages of total fatty acids in the hauled samples in relation to total lipid of samples and season are shown in Fig. 5. Samples with moderate to high lipid contents exhibited a consistent level of EPA and DHA, 4–8% and 7–10% of total fatty acids, respectively. However, as lipid content in the hauls decreased the relative proportions of n-3 LC-PUFA increased. In the final haul (213) of Fjord16, the EPA and DHA levels were 11.5% and 17.8% of total fatty acids, respectively. The variation of the relative contents of EPA and DHA in the mesopelagic fish, as a percentage of total lipid, with size and season is given in Fig. 6. For both fish species, there was a trend for EPA and DHA to decrease as fish size increased. Total EPA and DHA contents were rather stable for *M. muelleri* across the different size groups and there appeared to be a seasonal factor involved. This was particularly noticeable in the higher level of DHA in fish caught in Fjord15, in which the DHA level was very stable with only one sample of small fish having a very high DHA level. However, the EPA level always remained very low, at less than 4% of total fatty acids. In general, *B. glaciale* had higher levels of EPA and DHA when compared to *M. muelleri*, although they were also characterised by

Table 3
Lipid composition of the pooled samples. Fatty acids and fatty alcohols are given in mg.g lipid^{-1}.

	Fjord15				Fjord16			Ocean16				Mesopelagic fish[a]	
Trawl station	**430**	**431**	**434**	**447**	**186**	**188**	**191**	**208**	**209**	**211**	**213**	***M.muelleri***	***B. glaciale***
Total lipid (DW)	37.9	36.5	33.0	33.8	30.2	42.1	38.9	21.1	32.9	38.4	13.6	54.1 ± 4.83	47.5 ± 2.38
Percent distribution fatty acids – fatty alcohols													
Fatty acid	78.6	80.1	72.3	72.7	97.7	97.4	91.1	72.7	64.3	63.1	88.4	100	56.2 ± 0.17
Fatty alcohol	21.4	19.9	27.7	27.3	2.3	2.6	8.9	27.2	35.7	36.9	11.6	0	43.8 ± 0.17
Fatty acid (mg.g lipid^{-1})													
14:0	32.4	36.3	33.8	44.1	48.8	53.0	50.7	26.2	18.7	18.3	33.6	48.5 ± 1.42	26.2 ± 1.22
16:0	75.2	83.1	66.6	67.1	128.4	127.9	113.2	52.3	34.8	32.6	88.8	121.9 ± 1.67	28.7 ± 0.42
Total saturated[b]	***121.1***	***134.0***	***114.8***	***117.0***	***197.1***	***199.4***	***180.8***	***91.1***	***65.4***	***62.9***	***139.8***	***188.1 ± 1.07***	***65.7 ± 0.10***
16:1n-7	37.6	40.1	37.4	38.2	64.8	57.4	60.0	40.0	52.0	56.5	27.7	29.6 ± 0.53	46.9 ± 0.30
18:1n-9	84.2	90.1	80.6	82.3	92.5	67.9	78.4	101.4	126.3	138.4	82.9	87.5 ± 3.91	88.9 ± 6.44
20:1n-9	67.9	71.2	68.3	70.1	97.5	93.0	88.8	45.3	53.1	57.1	31.1	93.5 ± 1.78	48.8 ± 16.22
22:1n-11	104.3	110.5	103.6	105.1	170.7	166.5	149.9	49.0	50.5	56.8	38.0	197.1 ± 3.49	78.8 ± 26.30
Total monoenes[c]	***328.9***	***345.7***	***320.5***	***331.5***	***459.4***	***417.4***	***407.5***	***267.6***	***325.1***	***355.5***	***219.5***	***445.9 ± 2.71***	***292.4 ± 39.98***
18:2n-6	9.8	10.5	8.9	9.6	15.6	14.2	13.4	9.2	9.1	9.8	10.8	9.2 ± 0.18	7.6 ± 0.36
Total n-6[d]	***16.1***	***17.4***	***15.5***	***16.6***	***20.3***	***19.4***	***18.8***	***17.1***	***15.9***	***15.5***	***20.9***	***12.9 ± 1.71***	***11.7 ± 0.94***
18:3n-3	6.4	7.2	5.6	6.0	10.2	9.7	8.6	6.6	4.4	4.7	7.1	4.7 ± 0.03	4.7 ± 1.33
18:4n-3	15.2	17.0	14.0	14.8	23.2	23.5	20.4	18.6	11.5	12.4	19.7	6.3 ± 0.24	15.4 ± 4.67
20:5n-3	31.2	36.0	26.6	28.9	59.1	40.9	37.0	49.1	35.9	34.9	66.2	10.3 ± 0.05	16.0 ± 8.92
22:6n-3	50.7	64.5	44.4	48.4	76.8	53.4	49.9	73.0	44.3	44.9	102.2	22.2 ± 0.55	22.4 ± 12.23
Total n-3[e]	***110.7***	***132.6***	***96.9***	***104.8***	***181.4***	***132.5***	***121.9***	***155.1***	***101.9***	***102.9***	***204.2***	***48.6 ± 1.02***	***63.1 ± 29.05***
Total fatty acid[f]	***740.4***	***794.2***	***764.0***	***787.8***	***885.4***	***803.8***	***812.0***	***733.1***	***794.8***	***855.6***	***665.3***	***699.1 ± 4.00***	***438.9 ± 7.79***
Fatty alcohol (mg.g lipid^{-1})													
14:0	7.1	6.6	8.5	8.6	1.7	1.6	5.1	17.1	16.2	18.0	7.8	–	18.9 ± 3.76
16:0	33.5	34.3	43.7	44.0	6.9	6.1	17.5	43.4	35.8	37.5	23.3	–	81.2 ± 5.79
18:1n-9	12.8	14.4	16.7	17.1	1.5	1.3	4.7	25.2	20.9	23.3	13.4	–	36.9 ± 9.08
20:1n-9	26.5	26.3	36.9	37.9	1.3	1.2	10.5	32.5	55.7	61.9	7.1	–	57.1 ± 5.38
22:1n-11	68.9	68.2	95.1	96.5	10.0	8.9	28.8	69.8	123.7	140.7	21.1	–	122.2 ± 19.49
Total fatty alcohol	***158.1***	***158.8***	***211.6***	***215.2***	***23.5***	***21.1***	***72.2***	***199.2***	***283.8***	***316.2***	***77.4***	**-**	***93.5 ± 1.78***

[a] Based on means of 6 hauls of fish larger than 30 mm.
[b] Includes 15:0, 18:0, 20:0, 22:0 and 24:0.
[c] Includes 16:1n-9, 17:1, 18:1n-7, 20:1n-11, 20:1n-7, 22:1n-9 and 24:1n-9.
[d] Includes 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6 and 22:5n-6.
[e] Includes 20:3n-3, 20:4n-3, 21:5n-3 and 22:5n-3.
[f] Includes 16:2, 16:3 and 16:4.

having a much higher variation in total n-3 LC-PUFA content, regardless of fish size. For example, in large fish the DHA content varied from 2% to 14% and the EPA content from 1.5% to 7.5%. There was also a trend of lower EPA and DHA with increasing fish size.

The essential amino acid composition of the hauls and subsamples of the mesopelagic fish is shown in Table 4. The amino acid compositions of mixed hauls were relatively stable throughout season and between hauls, and were rather similar to those found in *M. muelleri*. In contrast, *B. glaciale* appeared to have higher levels of certain amino acids than *M. muelleri*, particularly valine, leucine, phenylalanine, methionine and lysine. When compared to known essential amino acid requirements (NRC, 2011), all hauls and species analysed contained far higher levels of all essential amino acids than the dietary requirements currently reported for Atlantic salmon.

The mineral composition of the hauls and subsamples of the mesopelagic fish are shown in Table 5. Calcium (Ca) and phosphorus (P) content of *M. muelleri* was 1.78 and 0.87% respectively compared to 1.2 and 0.83% for *B. glaciale*, respectively. The Ca:P ratio for *M. muelleri* was 1.2 and 1.46 for *B. glaciale*. These values are similar to those observed in the whole fish which were not subjected to fish meal processing (Lall, 1995). The range for Ca and P composition of the mixed hauls was 1 to 1.53% and 0.72–0.9% respectively. Magnesium (Mg) and sodium (Na) content of different hauls from spring and autumn ranged from 0.31 to 0.49% and 1.58–3.27% respectively. These values were higher than the Mg and Na contents determined in *M. muelleri* and *B. glaciale*. High concentrations of Na could likely be attributed to residual seawater in these samples during sample collection. Potassium content of all the hauls collected from the two different seasons as well as among the two mesopelagic fish species did not show large variation, ranging from 0.75 to 1.15%.

The trace element content of the different hauls from the autumn and spring seasons did not show any specific trend, although zinc and copper

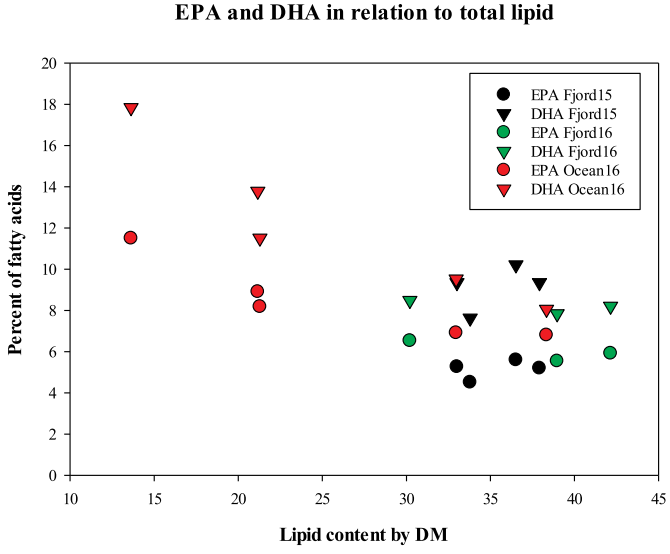

Fig. 5. The proportions of EPA (circle) and DHA (triangle) (percentage of total fatty acids) of the different mixed hauls in relation to total lipid contents (percent of dry matter) of the hauls.

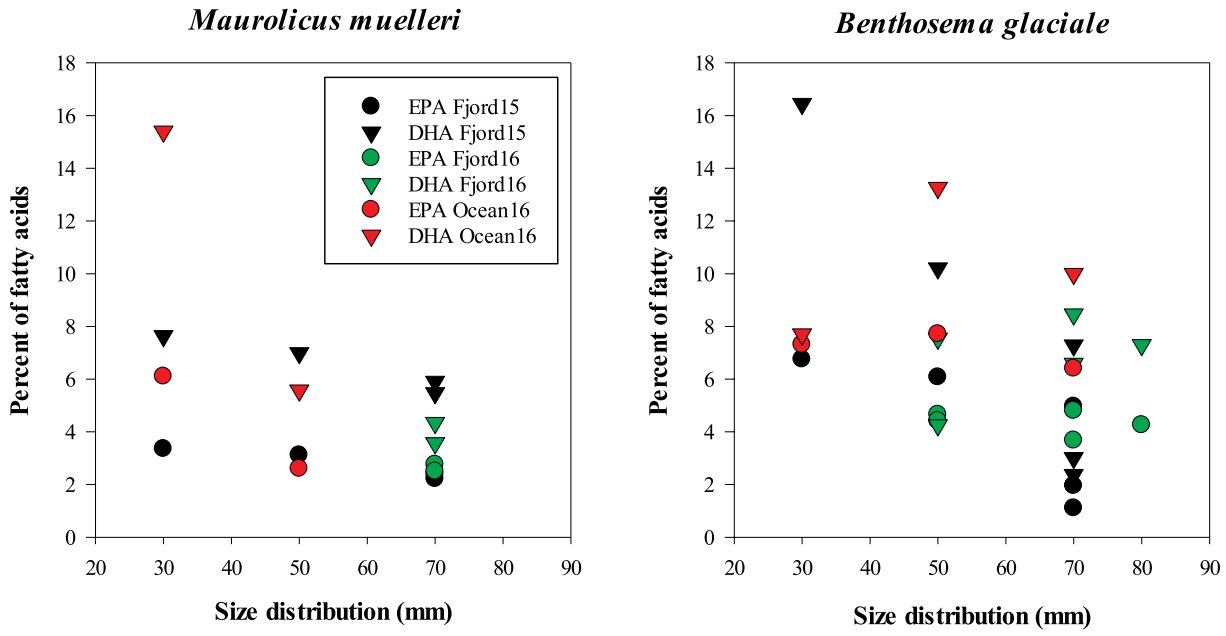

Fig. 6. The proportions of EPA and DHA (percentage of total lipids) in relation to cruise (season) and size distribution in the mesopelagic fish *Benthosema glaciale* and *Maurolicus muelleri*. Size distribution of fish is described in Materials and Methods section.

Table 4
Amino acid composition (% of dietary protein) of the catch.

	Fjord15				Fjord16			Ocean16				Mesopelagic fish		Requirement
Trawl station	430	431	434	447	186	188	191	208	209	211	213	*M.muelleri*	*B. glaciale*	At. Salmon[a,b]
Arginine	6.7	6.2	6.6	6.9	6.3	5.3	6.3	6.3	6.7	6.2	6.7	5.1 ± 0.24	5.7 ± 0.29	4.0 (1.8)
Histidine	2.0	1.8	2.0	2.0	2.2	1.8	2.2	1.9	2.0	1.8	1.9	1.9 ± 0.11	2.1 ± 0.14	1.8 (0.8)
Isoleucine	3.7	3.5	3.7	3.8	3.7	3.4	3.9	3.7	3.7	3.2	3.7	3.4 ± 0.20	3.8 ± 0.16	2.4 (1.1)
Leucine	6.6	6.1	6.5	6.7	6.6	6.1	6.9	6.4	6.2	5.5	6.4	6.4 ± 0.37	7.3 ± 0.32	3.3 (1.5)
Lysine	7.0	6.5	6.9	7.2	6.9	6.5	7.3	6.8	6.5	5.7	7.0	7.0 ± 0.44	8.2 ± 0.36	5.3 (2.4)
Methionine	2.3	2.6	2.3	4.3	2.3	2.3	2.4	2.3	2.1	2.0	2.4	2.5 ± 0.06	3.1 ± 0.30	1.6 (0.7)
Phenylalanine	3.9	3.7	3.8	4.1	4.1	3.6	4.0	3.8	3.7	3.7	4.0	3.7 ± 0.18	4.1 ± 0.18	2.0 (0.9)
Threonine	3.8	3.6	3.7	3.9	3.8	3.5	4.0	3.6	3.6	3.1	3.7	3.7 ± 0.23	4.0 ± 0.19	2.4 (1.1)
Tryptophan	1.0	1.0	1.0	1.0	1.0	1.1	1.1	1.0	1.0	0.8	1.0	1.0 ± 0.07	1.0 ± 0.04	0.7 (0.3)
Valine	4.3	4.0	4.2	4.3	4.2	4.1	4.5	4.2	4.2	3.7	4.1	4.2 ± 0.32	5.9 ± 0.29	2.8 (1.2)

[a] Amino acid requirement estimated from NRC values based on 44% of dietary protein.
[b] Values in parenthesis indicate NRC (2011) amino acid requirement values expressed as a percentage of dry diet.

Table 5
Macro minerals ($g.kg^{-1}$) and trace elements ($mg.kg^{-1}$) composition of the various catches.

	Fjord15				Fjord16			Ocean16				Mesopelagic fish	
Trawl station	430	431	434	447	186	188	191	208	209	211	213	*M.muelleri*	*B. glaciale*
Macro minerals ($g.kg^{-1}$)													
Calcium	12.4	15.3	13.4	14.5	14.5	11.1	10.7	13.6	11.1	9.9	17.8	10.3 ± 2.55	12.1 ± 0.63
Phosphorus	7.3	8.6	7.7	8.1	9.0	8.2	7.8	8.3	7.9	7.2	8.7	8.6 ± 1.37	8.3 ± 0.43
Magnesium	2.9	3.1	4.3	3.1	3.8	2.8	3.1	4.9	3.5	3.5	4.9	1.7 ± 0.15	1.2 ± 0.03
Sodium	17.6	15.8	28.2	15.8	23.3	17.6	19.4	32.7	20.9	22.8	19.2	5.8 ± 0.15	5.4 ± 0.08
Potassium	8.6	8.9	9.4	8.8	10.5	8.6	8.6	11.5	9.6	8.6	11.3	8.6 ± 1.23	7.5 ± 0.28
Trace elements ($mg.kg^{-1}$)													
Iron	48.1	55.7	83.4	52.2	34.5	448.1	65.8	214.9	50.9	56.5	52.2	44.3 ± 2.54	51.6 ± 9.29
Manganese	5.2	6.0	6.3	5.9	4.0	9.0	4.3	2.7	1.9	2.1	7.3	2.3 ± 0.11	2.8 ± 0.49
Zinc	83.6	85.3	105.9	56.1	29.3	7.0	10.6	18.1	30.1	40.2	22.5	34.4 ± 0.80	36.0 ± 3.79
Copper	16.0	14.9	18.7	19.6	1.0	1.1	1.1	1.0	1.0	0.8	1.0	1.0 ± 0.07	3.0 ± 0.41
Selenium	1.6	1.8	2.0	1.9	2.0	1.5	1.6	2.9	2.3	1.9	2.9	2.0 ± 0.21	1.7 ± 0.08
Nickel	1.1	1.3	1.1	2.0	0.7	1.2	1.0	1.0	1.2	1.1	0.9	0.9 ± 0.13	0.9 ± 0.13
Lead	1.1	2.1	0.6	0.4	0.3	0.3	0.7	0.3	0.3	0.4	1.7	0.3 ± 0.05	0.4 ± 0.07

were relatively high in all four of the haul samples collected in Fjord15. These values reflect the higher availability of food organisms, particularly invertebrates, available during this autumn sampling period and their accumulation in the body. These minerals are likely to be supplied by food organisms, especially invertebrates. Two hauls (188 and 208) showed higher concentrations of iron, with possible sources of this mineral including metal contaminants that could originate from the storage of samples in processing vessels where iron could potentially

leach into these products. A higher content has also previously been observed in certain fishery products. Other trace elements did not show a specific trend, although there were some variations between trawls and among the two fish species. Both nickel and lead concentrations were relatively low in all samples.

The heavy metal composition of the mixed hauls and selected mesopelagic fish are shown in Fig. 7. Mercury contents were far lower than the limit of 0.5 mg.kg^{-1} for all samples analysed. Arsenic was very low in mesopelagic fish, and borderline or, in a few cases, above the maximum limit (25 mg.kg^{-1}) for most hauls with the highest level found in haul 186 that was dominated by *Pasiphaea* sp. Cadmium contents were lower than the 2 mg.kg^{-1} limit in all samples except for the majority of hauls performed in Ocean16, where the highest level of 14 mg.kg^{-1} was found in haul 211. For the other hauls, levels of cadmium were 11 mg.kg^{-1} (haul 209), 2.9 mg.kg^{-1} (haul 208) and 0.64 mg.kg^{-1} (haul 213).

4. Discussion

The results of the present study clearly show that the mesopelagic layers in Norwegian fjords and off the coast of Norway are highly variable in their composition, with regards to both species and nutritive value. This means that it will be difficult to perform targeted fisheries aiming for certain species or a mixture of specific species. It also confirms previous observations that krill and shrimps are, by far, the predominating species in the mixed mesopelagic layers, up to 10 times more than the mesopelagic fish (Dalpadado et al., 1998; Skjoldal et al., 2004). As a possible feed source for aquaculture, this does not necessarily need to be seen as a disadvantage since krill and even copepods can be good protein and lipid sources for farmed fish (Olsen et al., 2006, 2010; Suontama et al., 2007b). Proteins and their constituent amino acids are essential components of fish feeds and the dietary protein requirements of salmonids and marine finfish for maximum growth typically range from 40 to 55%. The quality and nutritional value of most fish meal produced from anchovy, herring, capelin and menhaden are considered

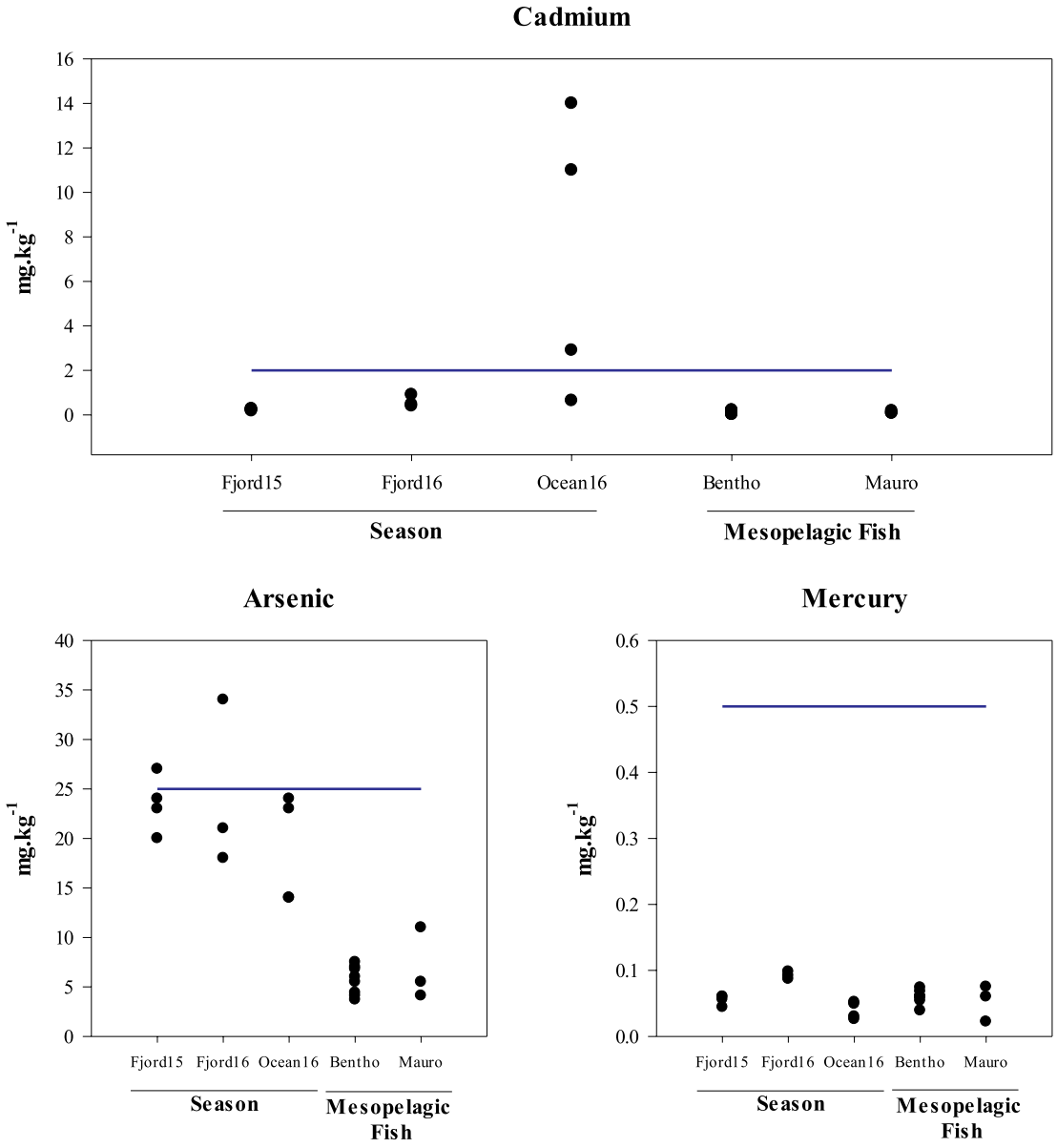

Fig. 7. Heavy metal contents (cadmium, arsenic and mercury; mg.kg^{-1}) of the hauls across seasons as well as for selected mesopelagic fish species, *Benthosema glaciale* and *Maurolicus muelleri*. The blue horizontal line indicates the maximum limits of 2, 25 and 0.5 mg kg^{-1} for cadmium, arsenic and mercury in Norwegian feeds, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

superior to major animal and plant proteins, because they are well balanced in all essential amino acids and their bioavailability is relatively high (Lall and Dumas, 2015; NRC, 2011). In addition, the essential amino acid composition of all the mesopelagic products sampled in the present study were found to be well balanced and would therefore be a valuable feed ingredient in salmon diets. Both mesopelagic fish, *M. mulleri* and *B. glaciale*, displayed an amino acid composition similar to herring and anchovy meal. In particular, the lysine, valine, methionine and phenylalanine content of *B. glaciale* was particularly high as compared to other products. These protein sources could be used in conjunction with other plant protein sources to improve the utilisation of common plant proteins currently used in salmon feeds.

One particular challenge with mesopelagic trawls as a raw feed material would be the high levels of chitin in some species. Chitin is poorly digested in Atlantic salmon and has been shown to reduce growth and feed conversion efficiency, whereas Atlantic cod (*Gadus morhua*) is able to utilise chitin due to the presence of chitinase in their digestive tract (Karlsen et al., 2017). Nevertheless, it is possible to remove chitin during the on-board processing stage.

One main reason to increase harvest of the mesopelagic level is the urgent need for the marine n-3 LC-PUFA, EPA and DHA. These are not synthesised by terrestrial oilseed crops and the production of algae or microbial sources in fermenters is still very expensive and not likely to supply sufficient quantities to satisfy the demand from the aquaculture industry (Sprague et al., 2017; Tocher et al., 2019). Consequently, lower trophic layer fishing has the potential to make large quantities of these fatty acids available to aquafeeds. If lipid and n-3 LC-PUFA were the main aim, then it would be preferable to target the mesopelagic fish such as *M. muelleri* and *B. glaciale*, since they can deposit up to 60% lipid dry weight (Falk-Petersen et al., 1986). The results from the present study support the current understanding of these fish as being rich in lipid, although the current data was consistently lower than the 60% reported previously. The lipid contents of the mesopelagic fish were generally in the range of 40–55% for medium to larger sized fish, with small and juvenile fish having lower lipid contents. However, the total lipid content in the mixed hauls was generally much lower than the level in the mesopelagic fish, generally in the range 20–40%. This was found to a consequence of the domination in the hauls of shrimps and krill that had much lower lipid contents. The lipid contents for these species were not detailed, although both *Sergestes* sp. and *M. norvegica* were found to have consistently lower levels of lipid (less than 20%, data not shown) despite previous studies reporting lipid levels of 50% or more for krill such as *M. norvegica*, and *Thysanoessa inermis* in autumn (Falk Petersen et al., 2000).

The fatty acid contents of the mixed hauls showed that they would be good sources of EPA and DHA, providing 80 $mg.g^{-1}$ or more of these fatty acids. There was a general tendency for the relative proportion of n-3 LC-PUFA to be lower when the lipid content of the haul was higher. This is largely expected since, in lean animals, the contribution of LC-PUFA from membrane phospholipids is more relatively important than when depot lipids (triacylglycerol or wax esters) increase (Olsen et al., 2010). Indeed, the content and composition of EPA and DHA is also dependent on diet preferences and availability. Therefore, feeding on lean prey animals will lead to a higher deposition of n-3 LC-PUFA in depot lipids than when feeding on prey with high lipid level, such as *Calanus*. In this respect, it was interesting that *M. muelleri* had higher levels than *B. glaciale* of gondoic (20:1n-9) and 22:1n-11 fatty acids that are known to be good markers of *Calanus* feeding (Petursdottir et al., 2008). It is however difficult to discriminate exactly the *Calanus* species that were targeted. The ratio of 20:1n-9/22:1n-11 was around 0.5 in *M. muelleri* whereas a diet consisting predominantly of *Calanus finmarchicus* should have given a ratio of 1:1 and preying on *C. glacialis* a ratio of 2:1. Based on a ratio of 0.5 would suggest that fish had been feeding upon *C. hyperboreus* (0.5), although the authors are not aware of the presence of this *Calanus* species in these locations. In contrast, *B. glaciale* had lower levels of the *Calanus* marker fatty acids, although the ratio of 20:1n-9/22:1n-11 was also around 0.5 suggesting that both species feed on similar *Calanus* species. However, it was also evident that PUFA content varied more in *B. glaciale* than *M. muelleri*, which could indicate that *B. glaciale* consume a wider range of prey species and participate more in opportunistic feeding when adapting to the regions.

A significant difference between *M. muelleri* and *B. glaciale* is that the depot lipid is stored as triacylglycerols in the former, whereas they are stored as wax esters in the latter. Wax esters are characterised by having a long-chain fatty alcohol esterified to a long-chain fatty acid. All the mixed haul samples from the present study contained some wax esters, thus it can be concluded that most of the wax esters in the hauls originated from the presence of *B. glaciale* in the samples. Since fatty alcohols are around 50% of the wax ester, it can be concluded that wax esters would contribute to between 40% to more than 70% of the lipid in the autumn samples and almost 90% in *B. glaciale*. This is a potential problem for use of these oils in salmon farming as salmon, like humans, cannot tolerate large amounts of wax esters in the diet. In humans, wax esters cause stomach cramps, diarrhea and even seborrhea (Olsen et al., 2010). In salmon, digestibility and feed utilisation will be reduced probably due to fatty alcohol accumulation, with growth reduced when the wax ester content exceeds 30–40% of the dietary lipid (Olsen et al., 2010). If this is also the case for wax esters specifically from *B. glaciale*, then the mesopelagic oils must be diluted with other oils before inclusion into fish feeds. Despite this, the contribution of n-3 LC-PUFA from mesopelagic oils will nevertheless be significant.

The minerals in fish meal and other fishery products contain higher mineral contents as well as a greater bioavailability compared to ingredients of plant and certain animal origins (NRC, 2011). The macro and trace element composition of all mesopelagic fish and other marine products analysed will be considered valuable feed ingredients in salmon diets. Indeed, most of these values observed 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. This can be explained due to the ingredients from the present study not undergoing standard processing techniques for meal and oil production, resulting in a relatively lower amount of bones being present as compared to processed fish meal. In certain fish meals, high amounts of Ca and P reduce the bioavailability of certain trace elements, particularly zinc. Thus, the lower P levels in these meals would have limited effects on trace element bioavailability but would still supply Ca and P for skeletal development of salmon. The products will also supply a good source of much needed organic selenium in salmonid feeds, since the increased inclusion of plant ingredients in salmonid feeds can lead to a lower uptake of selenium in the flesh (Betancor et al., 2016). Both the nickel and lead concentration was far below the maximum tolerable limit of these elements by fish (NRC, 2005). These ingredients show good potential as sources of dietary minerals and other nutrients and could therefore be used in conjunction with plant protein sources to improve the overall nutritional value of salmon feeds currently used.

It is well known that seafood is one of the main food sources for arsenic in the human diet. The majority of arsenic in seafood is organic with only a small fraction being in the highly toxic inorganic form. Arsenic levels are particularly high in many crustaceans and algae species, but is less common in fish (Borak and Hosgood, 2007; Taylor et al., 2017). Indeed, the high level of crustaceans in the mixed haul samples shows clearly that arsenic levels may cause some concern since many samples approached or even exceeded the upper legal limit as feedstuffs.

The high cadmium levels found in some of the hauls of the Ocean16, open sea, trawls probably relates to the increased occurrence of amphipods that are known to accumulate very high levels of cadmium (Rainbow, 1989; Ritterhoff and Zauke, 1998). The use of amphipod meal in, for example, weaning diets for cod has been shown to cause an increased development of deformities (Opstad et al., 2006). Because of the high levels of cadmium in amphipods, only a small biomass is sufficient to render the products useless as feed sources. This will necessitate targeted fisheries in areas without stocks of amphipods, or to

develop gear that would avoid fishing them.

5. Conclusion

The mixed mesopelagic layers in and off the coast of Norway are dominated by jellyfish, krill, shrimps and generally smaller amounts of the mesopelagic fish *M. muelleri* and *B. glaciale*. Protein content was typically high in samples with the amino acid composition deemed sufficient for use in feeds for farmed fish such as Atlantic salmon. In addition, all mesopelagic samples analysed showed that they were good sources of minerals that can be effectively used to reduce the inorganic mineral supplements in feeds for farmed fish. The majority of lipid from the mixed hauls originated from the mesopelagic fish as most krill and shrimp species appeared to be relatively lean. Nevertheless, the lipid composition was also shown to be a relatively good source of the marine n-3 LC-PUFA, EPA and DHA. However, many of the trawl hauls contained relatively high levels of wax esters that may be a problem if used in feeds for salmon as they have limited capacity to utilise them. Furthermore, many of the hauls from open water trawls contained mixtures of amphipods that caused the cadmium level to exceed the maximum allowable level in feedstuffs. This is a major challenge that needs to be addressed. Likewise, the high level of crustaceans in many samples led to arsenic levels being borderline and, in a few cases, higher than the upper limit for use in feeds. Reductions in crustacean mix into the hauls appears to be the only viable option to reduce these levels. In contrast, mesopelagic fish had low levels of all heavy metals.

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