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Temporal variations in the nutrient content of Norwegian farmed Atlantic salmon (*Salmo salar*), 2005–2020

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ABSTRACT

The changes in the feed of farmed Atlantic salmon (*Salmo salar*) towards a more plant-based diet affect the nutritional value of the fillets. By compiling the contents of a range of nutrients in 1108 samples of Norwegian farmed Atlantic salmon collected between 2005 and 2020, we found that the median contents of eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) have decreased by > 60%. However, farmed Atlantic salmon remains a considerable source of EPA and DHA, with one and two portions being sufficient to meet the weekly adequate intake of EPA and DHA for adults (175 g) and two-year-olds (80 g), respectively. Farmed Atlantic salmon also remains a considerable source of protein, selenium, vitamin B_{12}, and vitamin D_3. Together, we demonstrate that farmed Atlantic salmon can contribute substantially to the nutrient intake of the consumers. These data are important for the Norwegian food composition table and future risk–benefit assessments on fatty fish consumption.

1. Introduction

According to the recent biannual "The State of World Fisheries and Aquaculture" report by the Food and Agriculture Organization of the United Nations (FAO) (FAO, 2020), global aquaculture production has been the fastest growing food production sector for the past few decades, currently providing more than half of the global supply of fish available for human consumption. Simultaneously, global capture fisheries production has reached a plateau. In order to keep pace with the continuously growing global population and the increase in demand for fish and seafood products, a greater proportion of fish are being farmed. Atlantic salmon (*Salmo salar*), one of the main fish species in European aquaculture, represent an increasingly popular species among consumers worldwide (FAO, 2020). The five leading salmon farming countries include Norway, Chile, Scotland, Canada, and the Faroe Islands; with Norway as the largest producer with a production share of 55% (Iversen, Asche, Hermansen, & Nystøyl, 2020). At present, it is estimated that 14 million meals of Norwegian farmed Atlantic salmon are consumed every single day across the world (Norges sjømatråd [Norwegian Seafood Council], 2020).

Traditionally, farmed Atlantic salmons were fed a diet with high levels of marine ingredients, such as fish meal and fish oil, generally derived from pelagic fisheries (Sissener et al., 2013; Ytrestøyl, Aas, & Åsgård, 2015). However, due to the rapid growth of the aquaculture industry and pressure on wild fish stocks, limited access and volatile prices of marine ingredients combined with growing demands for sustainability have led to a considerable shift in the composition of the diet of farmed Atlantic salmon (FAO, 2020; Ytrestøyl et al., 2015). Since the 1990s, an increasing part of marine ingredients has been replaced by a higher proportion of plant ingredients of terrestrial origin, such as rapeseed oil and soy protein concentrate (Aas, Ytrestøyl, & Åsgård, 2019; Sissener et al., 2013). Experimental feeding trials have demonstrated that lower proportions of marine ingredients have caused reduced levels of the marine long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFA) eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) in the fillets of Atlantic salmon (Bell,

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Henderson, Tocher, & Sargent, 2004; Torstensen et al., 2005).

Fish in general is a rich source of several key nutrients such as high-quality animal protein, EPA and DHA, and numerous micronutrients, including vitamin A, vitamin B_{12}, vitamin D, iodine, and selenium (FAO/WHO, 2011; Hicks et al., 2019). Intake of n-3 LC-PUFA is associated with a reduced risk of coronary heart disease mortality in adults and improved functional outcomes of neurodevelopment in children when seafood, and particularly fish, is consumed during pregnancy (EFSA, 2014; FAO/WHO, 2011). Although recommendations for the daily intake of EPA and DHA vary globally (Global Organization for EPA and DHA Omega-3, 2014), most dietary guidelines suggest a weekly consumption of at least two portions of fish, of which one should be fatty, for the general population (European Commission, 2020). Fish is also an important provider of other essential nutrients that may contribute considerably to the nutrient intake of the consumers. For instance, very few foods contain vitamin D naturally, and fillets from fatty fish such as salmon, mackerel, and herring are considered to be among the best sources (Nøstbakken et al., 2021). Furthermore, foods from the marine environment are among the richest natural food sources of the trace element iodine (Dahl, Johansson, Julshamn, & Meltzer, 2004; Nerhus et al., 2018). Both iodine and selenium are provided to the aquatic feed through fishmeal, and a general trend of declining iodine and selenium contents in Norwegian fish feed has been reported for the years 2000–2010 (Sissener et al., 2013). It has been experimentally shown that mean iodine content in the fillets of Atlantic salmon correlated with dietary iodine supplementation of the salmon feed (Julshamn, Maage, Waagbø, & Lundebye, 2006), but it is not yet known if and how increased use of plant feed ingredients may have influenced the iodine content in the salmon fillets.

The shift from marine to increased use of plant ingredients in fish feed may affect the nutritional content of the fish fillets and thus the nutrient intake of the consumers. Previous studies have examined the contaminant content in Norwegian farmed Atlantic salmon over a 13-year period (Nøstbakken et al., 2015) and the difference in the contaminant content and the fatty acid composition of wild and farmed Norwegian Atlantic salmon (Lundebye et al., 2017). However, the trends of nutrient content in Norwegian farmed Atlantic salmon over time have not been specifically examined. Thus, in the present paper, analytical data on total fat, fatty acids, protein, vitamin B_{12}, vitamin D_3, iron, iodine, and selenium in fillets of Norwegian farmed Atlantic salmon are presented for a time period of 15 years, from 2005 to 2020. Further, the contents of selected micronutrients in farmed Atlantic salmon from 2005 and 2020 were examined in view of defined nutrient recommendations for women of reproductive age and two-year-old children.

2. Material and methods

2.1. Fish samples

The present study comprises 1108 samples of Norwegian farmed Atlantic salmon (*Salmo salar*) sampled between 2005 and 2020. The samples of farmed Atlantic salmon were collected year-round by inspectors from the Norwegian Food Safety Authority at various fish farms along the coast of Norway as part of the European Union's (EU) monitoring programme, as specified in the EU council directive 96/23 (Council of the European Union, 1996), and through additional projects at the Institute of Marine Research (IMR). The salmon were slaughtered in accordance with current regulations for commercial slaughter methods, and the sampling was randomised with regard to season and regions across the whole of coastal Norway. Between five and ten fish samples of market-size were collected from each fish farm and transported frozen (-20 °C) to the IMR laboratories where sample preparation was performed. A standardised muscle sample (the Norwegian Quality Cut (NQC); (Norwegian standard 9401.E, 1996)) was collected from each fish sampled between 2011 and 2020; this encompassed discarding of the spine, bones, and skin, and inclusion of the subcutaneous fat that is commonly consumed. From the salmon sampled in 2005, 2006, and 2010, a standardised fillet sample of the whole side of the fish was collected by making a diagonal cut behind the pectoral fin towards the head, before cutting along the backbone down to the tail of the fish, also including the subcutaneous fat and removal of the spine, bones, and skin. An overview of the samples and their physical parameters is presented in Table 1. Individual samples were homogenised using a conventional food processor and thereafter freeze-dried (Labconco FreeZone 18 L, mod. 7750306, Kansas City, MO, USA). The freeze-dried material was homogenised once more to a fine powder which was utilised for all analyses, except for lipid and vitamin analyses where wet sample material was utilised (sample material prior to freeze-drying). The wet samples were kept frozen after homogenisation at −80 °C pending analysis, whereas the freeze-dried samples were kept dry pending analyses (room temperature).

2.2. Analytical methods

Analyses were performed at the IMR laboratories in Bergen, Norway, using methods accredited to the ISO/IEC 17025:2005 standard (the laboratories were accredited to ISO/IEC 17025:1999 in years 2005–2006). The analytical methods are regularly verified by participation in national and international interlaboratory proficiency tests, and Certified Reference Materials (CRM) are analysed at least once a year to check the accuracy and precision of the methods. Furthermore, self-produced internal control materials, or reference materials, are included in each sample run for quality control. Details on the analytical methods, CRM, internal control materials, limits of quantification (LOQ), and the uncertainty of the measurements are described by Reksten et al. (Moxness Reksten et al., 2020). The number of samples (n) per analyte per year is provided in Tables 2 and 3. The nutrients analysed varied from year to year due to budget constraints; some nutrients were analysed every year, whereas others more irregularly. No data are available for the years 2007–2009.

2.2.1. Protein, total fat, and fatty acid composition

For the determination of the total lipid content, the homogenised samples were extracted with isopropyl alcohol in ethyl acetate and filtered before the solvent evaporated and the fat residue was weighed. The method is based on a Norwegian Standard (Norwegian Standard 9402, 1994). The protein content was determined by burning the freeze-dried sample material in pure oxygen gas in a combustion tube (from 2005 to 2012: Leco FP-528 Nitrogen Determinator, Leco Corporation, Saint Joseph, MI, USA; from 2013 to 2017: Vario MACRO Cube Analyzer, Elementar Americas, Ronkonkoma, NY, USA; from 2018: Leco FP-628 Nitrogen Determinator, Leco Corporation, Saint Joseph, MI, USA). The nitrogen was detected with a thermal conductivity detector and the content of nitrogen was calculated from an estimated average of 16% nitrogen per 100 g protein using the following formula: nitrogen g/100 g × 6.25 = protein g/100 g. The method is accredited according to AOAC Official Methods of Analysis (AOAC, 1995). The fatty acid composition of the homogenised samples was analysed as previously described (Moxness Reksten et al., 2020). Briefly, the lipids from the samples were extracted by adding chloroform/methanol (2:1, v/v). After filtering, the lipids were saponified and methylated, and the methyl ester was extracted with hexane. Methyl esters were separated using various gas chromatographs: from 2005 to 2012, a Thermo Finnigan Trace GC2000 (Thermo Scientific Waltham, MA, USA) was used, from 2005 to 2020, a Perkin Elmer Auto System XL2000 (Perkin Elmer, Waltham, MA, USA) was used; whereas from 2017 to 2020, a Bruker Scion 435 GC (Scion Instruments, Livingston Scotland, UK) was used. Cold on column injection with a Flame Ionization Detector (FID) and a 50 m CP-sil 88 fused silica capillary column (id: 0.32 mm) (Chromopack Ltd., Middelburg, The Netherlands) was used with all the gas chromatographs. Fatty acids were identified by retention time using standard mixtures of methyl esters (Nu-Chek, Elysian, MN, USA), thus

Table 1
Overview of the total number of samples (n) per year and physical parameters of the samples of Norwegian farmed Atlantic salmon (*Salmo salar*) collected between 2005 and 2020.

Year	n[a]	Type of tissue	Weight (g) Mean ± SD	Min - Max	Length (cm) Mean ± SD	Min - Max	K-factor[b] Mean ± SD	Min - Max
2005	50[c]	Fillet	4037 ± 928	2520–6590	67 ± 6	56–80	1.4 ± 0.4	0.8–2.7
2006	40	Fillet	2628 ± 1187	940–5800	58 ± 9	41–78	1.3 ± 0.2	0.6–2.0
2010	33	Fillet	4009 ± 1168	997–6262	68 ± 7	48–83	1.3 ± 0.2	0.9–2.1
2011	100	NQC	4324 ± 1218	2150–9200	73 ± 7	57–93	1.1 ± 0.2	0.7–1.5
2012	100	NQC	N/A[d]	N/A[d]	N/A[d]	N/A[d]	N/A[d]	N/A[d]
2013	100	NQC	3647 ± 905	1300–5800	68 ± 6	53–79	1.1 ± 0.1	0.6–1.5
2014	100	NQC	4751 ± 1109	1877–8184	71 ± 5	55–84	1.3 ± 0.2	1.0–1.8
2015	100	NQC	4103 ± 1069	1813–8155	70 ± 5	57–83	1.2 ± 0.2	0.8–1.6
2016	100	NQC	3652 ± 877	1500–5800	69 ± 5	55–81	1.1 ± 0.2	0.7–1.4
2017	100	NQC	3656 ± 1088	900–6300	69 ± 7	51–82	1.1 ± 0.2	0.6–1.5
2018	100	NQC	3845 ± 1147	1000–7600	69 ± 5	53–83	1.1 ± 0.2	0.6–1.5
2019	105[e]	NQC	3874 ± 992	1008–6540	69 ± 5	54–80	1.2 ± 0.2	0.6–2.1
2020	80	NQC	3818 ± 874	1850–5630	69 ± 6	52–81	1.2 ± 0.2	0.8–1.6

Abbreviations: NQC: Norwegian Quality Cut (Norwegian standard 9401.E, 1996).
SD: standard deviation.
[a] The number of samples varied for different analytes. The n described in this table reports the total number of samples collected from the various fish farms each year
[b] K-factor, or condition factor, refers to the numerical value of a salmonid that reflects its condition. A K-value of < 1.0 indicates a poor fish (long and thin), a K-value of 1.2 indicates a fair fish, whereas a K-value of 1.4 indicates a good (well-proportioned) fish (Barnham & Baxter, 1998).
[c] Physical parameters are only available for 46 samples.
[d] Physical parameters are not available.
[e] Physical parameters are only available for 100 samples.

determining the fatty acid composition (area %). The amount of fatty acids per gram sample material was calculated using 19:0 methyl ester as an internal standard.

2.2.2. Vitamins

Vitamin B_{12} (cobalamin) was extracted from the sample (autoclaving in acetate buffer) and mixed with growth medium, before the microorganism *Lactobacillus delbrueckii* (ATCC 4797) was added and the sample was incubated at 37 °C for 22 h. The vitamin content was calculated by comparing the growth of the organism to known standard concentrations with turbidimetric reading (Optical Density, OD, v/575 nm) (Angyal, 1996). The content of vitamin D_3 (cholecalciferol) was determined by saponifying the sample material, extracting the unsaponifiable material and purifying it on a preparative high-performance liquid chromatograph (HPLC) column. The fraction containing vitamin D_3 was pooled (normal phase) and injected on a HPLC column (reverse phase). The content of vitamin D_3 was then determined by an ultraviolet (UV) detector, using vitamin D_2 (ergocalciferol) as an internal standard (CEN, 2009).

2.2.3. Minerals

Total contents of iron and selenium were quantified by inductively coupled plasma-mass spectrometry (ICP-MS) after wet digestion in a microwave, as described by Julshamn et al. (Julshamn et al., 2007). For analyses performed from 2005 to 2013, an Agilent 7500CE ICP-MS (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a Setac 5000 autosampler was used, whereas for analyses performed from 2014 to 2020, an iCapQ ICP-MS (ThermoFisher Scientific, Waltham, MA, USA) equipped with an FAST SC-4Q DX autosampler (Elemental Scientific, Omaha, NE, USA) was used. The iodine content was also determined using ICP-MS, however, tetra methyl ammonium hydroxide (TMAH) and water were added to the samples before extraction. Internal standards and external linear calibration curves were used to quantify the contents of the elements: for iron and selenium, scandium (Sc) was used, whereas for iodine, tellurium (Te) was used as the internal standard.

2.3. Statistical analyses and presentation of analytical data

All statistical analyses of the data and graphs presented as temporal trends were created using the statistical programming language R (version 4.0.3) running in RStudio (version 1.2.5042, © 2009–2020, RStudio Inc., Boston, MA, USA), whereas other graphs were compiled using GraphPad Prism (version 8.3.0, 2019, GraphPad Software, San Diego, CA, USA). The normality of the data and the normality of the residuals of the data were assessed using histograms and Shapiro-Wilk test. Due to the lack of normality, the non-parametric Kruskal-Wallis one-way analysis of variance followed by multiple Wilcoxon *post hoc* tests on all possible pairwise comparisons were used to assess significant differences between the years. A significance level of $p \leq 0.05$ was applied to all statistical tests performed. To examine correlation between various parameters, correlation analyses using Spearman's rank correlation coefficient were performed. Local polynomial regression fitting (loess), as described by Jacoby (Jacoby, 2000), was used for the assessment of temporal trends for the various analytes. For values below the limit of quantification (LOQ), values between zero and the LOQ value were randomly generated from an appropriate distribution and imputed into the dataset as a replacement (the fill-in approach). Nutrients with > 30% of the values < LOQ were excluded and not reported due to the uncertainty connected to high levels of censored values (Lubin et al., 2004); this included 12 of the 45 fatty acids analysed. Results are presented as medians and interquartile ranges (IQR) reported to the same units of expression and number of significant digits as advised in the FAO guidelines "Food Composition Data: Production, Management, and Use" (Greenfield & Southgate, 2003). In the tables, results are presented as both medians with IQR and means ± standard deviations (SD) in addition to the range between the minimum and maximum values. Significant differences between data in tables are indicated by different superscript lettering.

2.4. Calculations of potential contribution to recommended nutrient intakes

The median nutrient values for the salmon sampled in 2005 and 2020 were compared with the recommended nutrient intakes for non-pregnant, non-lactating, healthy women of reproductive age (pre-menopausal) and two-year-old children for the micronutrients analysed; EPA + DHA, vitamin B_{12}, vitamin D_3, iron, iodine, and selenium. For vitamin B_{12}, vitamin D, iron, iodine, and selenium, dietary reference intakes (RI) described in the Nordic Nutrition Recommendations, 2012 (NNR 2012) (Nordic Nutrition Recommendations 2012, 2014) were used (supplementary material, Table A.1). The RI is defined as the intake level sufficient to meet the daily requirements for most healthy individuals in a specific population and is based on an estimated average nutrient requirement (EAR) plus two SD above the mean (Nordic Nutrition Recommendations 2012, 2014). For EPA + DHA, recommendations of an adequate intake (AI) of 250 mg per day for adults and children aged ≥ two years were used in the calculations, as advised by the European Food Safety Authority (EFSA) (EFSA, 2010), as the NNR 2012 only specifies recommendations for the total intake of omega-3 fatty acids (≥1 energy percent (E%)) and not for single fatty acids (Nordic Nutrition Recommendations 2012, 2014). As opposed to RI, AI is a dietary recommendation used when there is insufficient scientific evidence to determine the EAR (also termed "average requirement", or AR, by the EFSA), and thus the RI. The AI is the average nutrient level consumed daily by a typical healthy population that is assumed to be adequate to meet the population's needs (EFSA, n.d.). A standardised portion size of 175 g raw salmon fillet, as described in the report "Weights, measures, and portion sizes of foods" (Østerhold Dalane, Martinsen Bergvatn, Kielland, & Hauger Carlsen, 2015), was used as the reference portion size for adults in the calculations of the current study. For children, 80 g raw salmon fillet, or approximately 50% of the portion size of adults, were used as no standardised portion sizes for fish and seafood for this age group currently exist in Norway.

3. Results

3.1. Correlation between physical parameters and various nutrients

Possible associations between weight, length, the content of EPA and DHA, total fat, total protein, and the analysed micronutrients were examined using Spearman's correlation analysis. There was a moderate, but significant correlation between the total fat content and the EPA and DHA content ($r = 0.53$ and 0.38, respectively, $p < 0.001$), and the content of EPA showed a strong positive correlation with the content of DHA ($r = 0.74$, $p < 0.001$). However, neither a correlation between the total fat content and the vitamin D_3 content, nor a correlation between the total fat content and any of the other micronutrients analysed were discovered. A moderate, yet significant, negative correlation was observed between the EPA and DHA content and the protein content ($r = -0.35$ and $r = -0.28$, respectively, $p < 0.001$). As for the other micronutrients analysed, a weak, but significant positive correlation was observed between the selenium and vitamin B_{12} content ($r = 0.36$, $p < 0.001$) and between the iodine and vitamin B_{12} content ($r = 0.27$, $p < 0.001$), whereas no correlation was discovered between any of the other micronutrients analysed.

3.2. Content of protein and total fat

The protein content was analysed in 2005, 2006, 2011–2013, 2015, and in 2017–2020. A total of 747 samples were analysed during these years. The median protein content ranged from 18 to 20.1 g/100 g, with the highest content reported in 2011 and the lowest content reported in 2015 (supplementary material, Table B.1). From 2005 to 2020, a general upwards trend in the protein content is observed, except for a slight decline in 2015 (Fig. 1). Nevertheless, the protein content has remained generally stable over the years, although some significant differences were observed between years.

The total fat content was analysed in a total of 1078 samples in 2005 and 2006 and thereafter every year from 2010 to 2020. The median fat

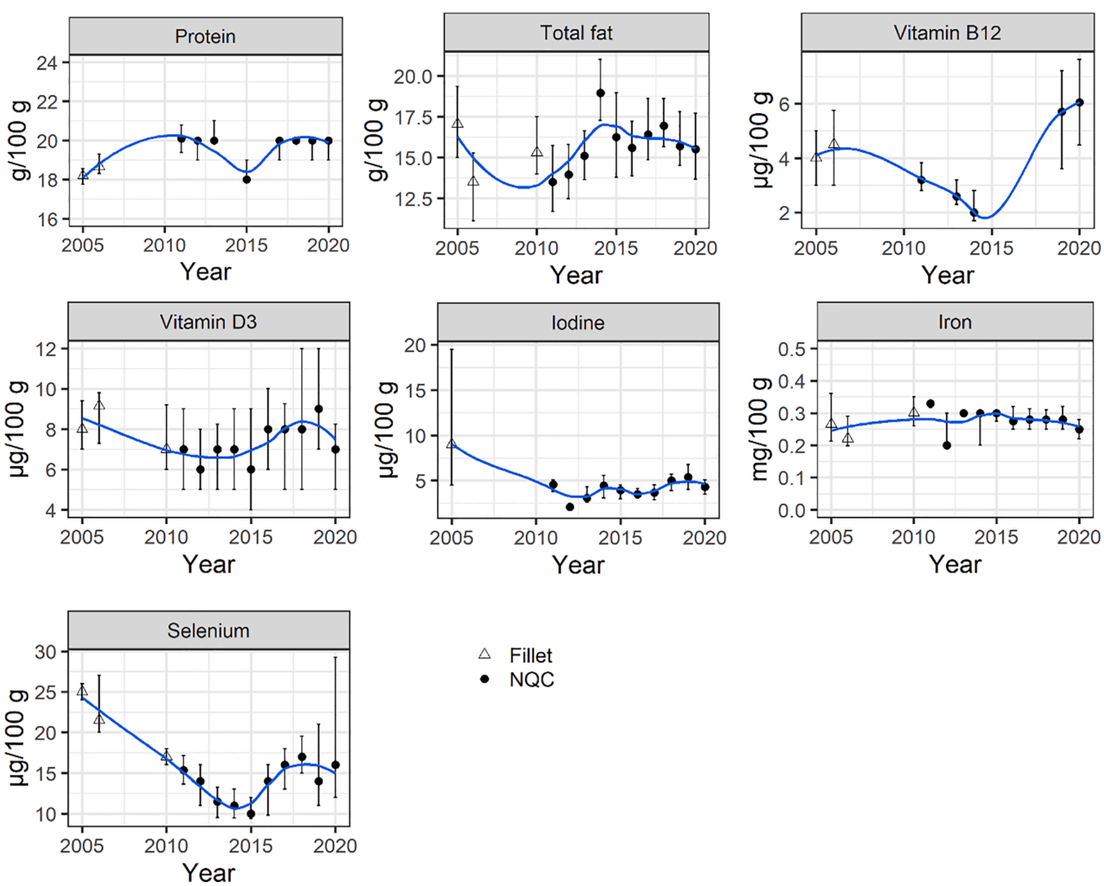

Fig. 1. Temporal variations in the content of total protein, total fat, vitamin B_{12}, vitamin D_3, iodine, iron, and selenium in the fillets of Norwegian farmed Atlantic salmon (*Salmo salar*) from 2005 to 2020. The data are presented as medians and interquartile ranges (IQR), where the blue line illustrates the temporal trend of the nutrient content over the years as analysed using local regression analysis.

content ranged from 13.5 g/100 g in 2006 and 2011, to a peak content of 19.0 g/100 g in 2014 (supplementary material, Table B.1). The fat content significantly decreased ($p < 0.001$) from 2005 to 2013, before reaching a peak in 2014 and then flattening out towards 2020 with a median content of roughly 15–17 g/100 g for the past five years (Fig. 1).

3.3. Fatty acids

The fatty acid composition was analysed in a total of 1075 samples of farmed Atlantic salmon from 2005 and 2006, and thereafter every year from 2010 to 2020 (supplementary material, Table C.1 and D.1). For 12 fatty acids (6:0, 8:0, 10:0, 12:0, 14:1n-9, 16:3n-3, 16:4n-3, 20:3n-3, 20:3n-9, 22:4n-6, 24:0, 24:6n-3), a large part of the samples were below the LOQ (>30%; LOQ = 0.01 mg/g), thus, the data for these fatty acids are not presented. The analytical data and temporal trends of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), total omega-3 fatty acids, sum EPA + DHA, docosapentaenoic acid (DPA, 22:5n-3), α-linoleic acid (ALA, 18:3n-3), total omega-6 fatty acids, and linoleic acid (LA, 18:2n-6) are shown in Table C.1 in the supplementary material and in Fig. 2, respectively, whereas temporal trends and analytical data of other individual fatty acids are presented in the supplementary materials (Figure E.1 and Table D.1, respectively).

There has been a significant decrease ($p < 0.001$) in the overall content of omega-3 fatty acids from 2005 to 2020, whereas the content of total omega-6 fatty acids has significantly increased ($p < 0.001$). This trend is particularly noticeable from 2010 onwards as the content of total omega-3 fatty acids decreased by approximately 35% from 2010 to 2011. From 2005 to 2020, the content of total omega-3 fatty acids decreased by 39%, whereas the content of total omega-6 fatty acids has more than doubled during the past 15 years. The contents of EPA + DHA have significantly decreased ($p < 0.001$) by > 60% from 2.75 g/100 g in 2005 to 1.07 g/100 g in 2020. Nevertheless, during the past five years, the total PUFA content has remained stable as the contents of both total omega-3 and total omega-6 fatty acids have remained stable at around 2.50–2.70 g/100 g, whereas the EPA + DHA contents have remained stable at around 1.03–1.30 g/100 g since 2011. Furthermore, slightly increasing levels of MUFA and decreasing levels of SFA are evident.

3.4. Content of vitamin B_{12} and vitamin D_3

A total of 482 samples were analysed for vitamin B_{12} in years 2005, 2006, 2011, 2013, 2014, 2019, and 2020. The vitamin B_{12} content peaked in 2020 with a median value of 6.1 μg/100 g, whereas the lowest content of 2.0 μg/100 g was reported in 2014 (supplementary material, Table B.1). From 2005 to 2014, the content significantly decreased ($p < 0.001$), before significantly increasing ($p < 0.001$) and reaching a peak in 2019 and 2020 (Fig. 1). Vitamin D_3 was analysed in samples from 2005 and 2006, and thereafter in samples collected every year from 2010 to 2020. A total of 1095 samples were analysed, of which three samples (0.3%) were < LOQ (0.01 mg/kg wet weight; two samples in 2017 and one sample in 2018). The vitamin D_3 content has remained relatively stable over the years with median values ranging between 6.0 and 9.2 μg/100 g (Fig. 1). The content reached a peak in 2006 with 9.2 μg/100 g, whereas the lowest value of 6.0 μg/100 g was reported in 2012 and 2015 (supplementary material, Table B.1). From 2005 to 2015, the content significantly decreased ($p = 0.001$), before increasing from 2016 onwards. In 2020, the median content decreased to a similar

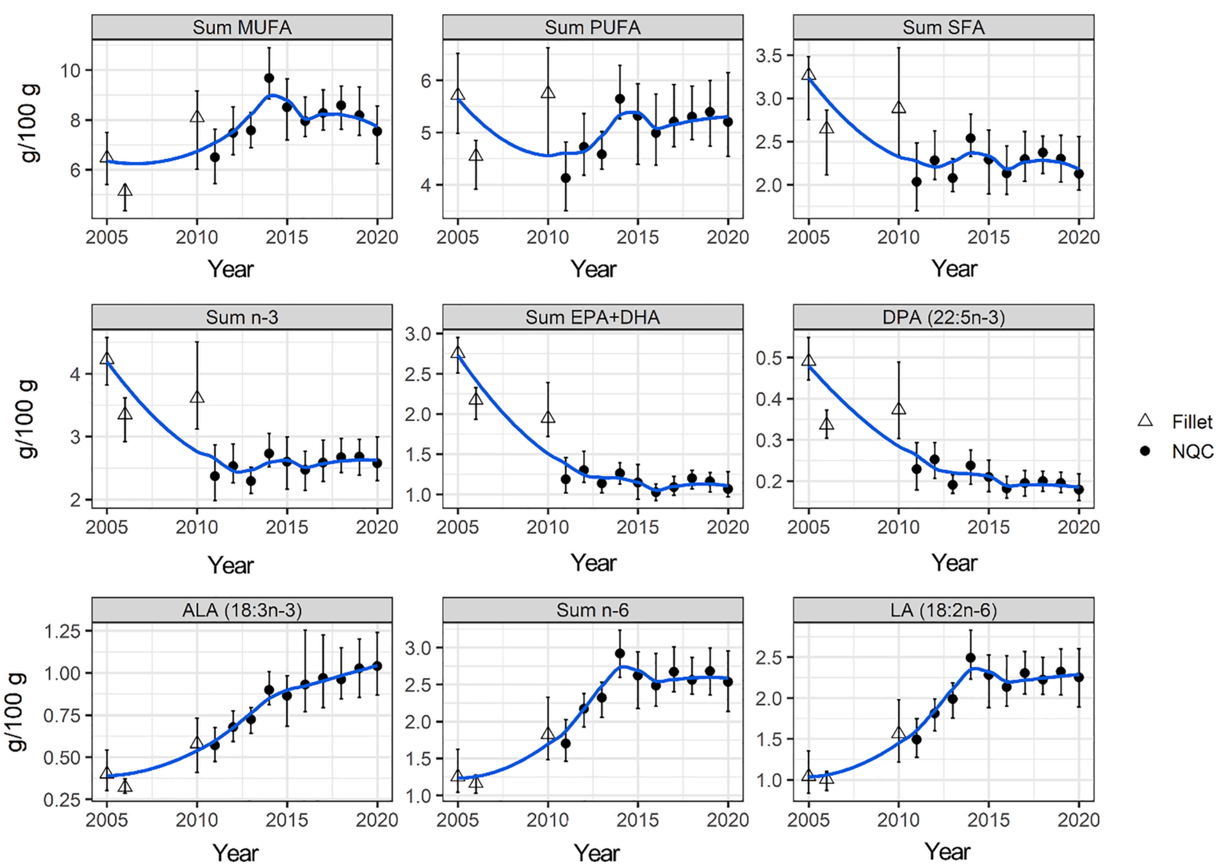

Fig. 2. Temporal variations in the fatty acid composition (g/100 g wet weight) of fillets of Norwegian farmed Atlantic salmon (*Salmo salar*) from 2005 to 2020. The data are presented as medians and interquartile ranges (IQR). The blue lines illustrate the time trends of the contents of various fat components over the years as analysed using local regression analysis. **Abbreviations**: ALA: alpha-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; LA: linoleic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.

level to that between 2010 and 2015.

3.5. Content of iodine, iron, and selenium

Iodine was determined in a total of 1005 samples, whereas 1088 and 1003 samples were analysed for selenium and iron, respectively. The iodine content was analysed in samples from 2005 and in samples collected every year between 2011 and 2020, whereas the selenium and iron contents were analysed in 2005 and 2006, and then every year from 2010 to 2020. For iodine, the measured content in three of the total 1027 samples (0.3%) were < LOQ (0.01 mg/kg wet weight; one sample in 2013 and two samples in 2017), whereas all values were > LOQ for selenium and iron. The temporal changes of the iodine, selenium, and iron contents are presented in Fig. 1. The iodine content was highest in 2005 with a median value of 9.0 μg/100 g before significantly decreasing ($p < 0.001$) and reaching the lowest content of 2.1 μg/100 g in 2012 (supplementary material, Table B.1). From 2011 to 2020, the median content has remained relatively stable between 2.1 and 5.4 μg/100 g. Similar to iodine, the selenium content was highest in 2005 with 25 μg/100 g, before significantly decreasing ($p < 0.001$) and reaching the lowest content of 10 μg/100 g in 2015. From 2015 to 2020, the selenium content has significantly increased ($p = 0.001$). The content of iron has remained relatively stable between 0.20 and 0.30 mg/100 g, although some significant differences between years were observed.

3.6. Contribution to recommended intakes of nutrients

The measured micronutrient content in farmed Atlantic salmon from 2005 and 2020 was evaluated in the context of recommended nutrient intakes for women and children (Fig. 3). A portion size of 175 g raw salmon fillet was used for women, whereas the portion size for two-year-old children was estimated to be 80 g. The daily recommendations for women and two-year-olds were met by considerably >100% for EPA + DHA when either 2005 or 2020 contents were used, however, no tolerable upper intake level (UL) for EPA and/or DHA has been set by any authoritative body (EFSA, 2012). One portion of farmed Atlantic salmon from 2005 was able to provide approximately 1920% and 880% of the daily recommendations for women and two-year-olds, respectively, whereas one portion from 2020 was able to provide approximately 750% and 340% of the recommendations, respectively. Furthermore, when assessing the number of portions required to obtain a weekly intake of 1.75 g EPA + DHA (250 mg daily × 7 days), one portion of farmed Atlantic salmon from all years analysed was found to be more than adequate to meet the recommendations for adults (Fig. 3C). For two-year-old children, a single serving of 80 g would have been sufficient to meet the recommended weekly intake level in 2005, 2006, and 2010, whereas from 2011 onwards approximately two portions are required.

For vitamin B_{12}, with no established UL (Nordic Nutrition Recommendations 2012, 2014), 350% of the RI for women and 400% of the RI

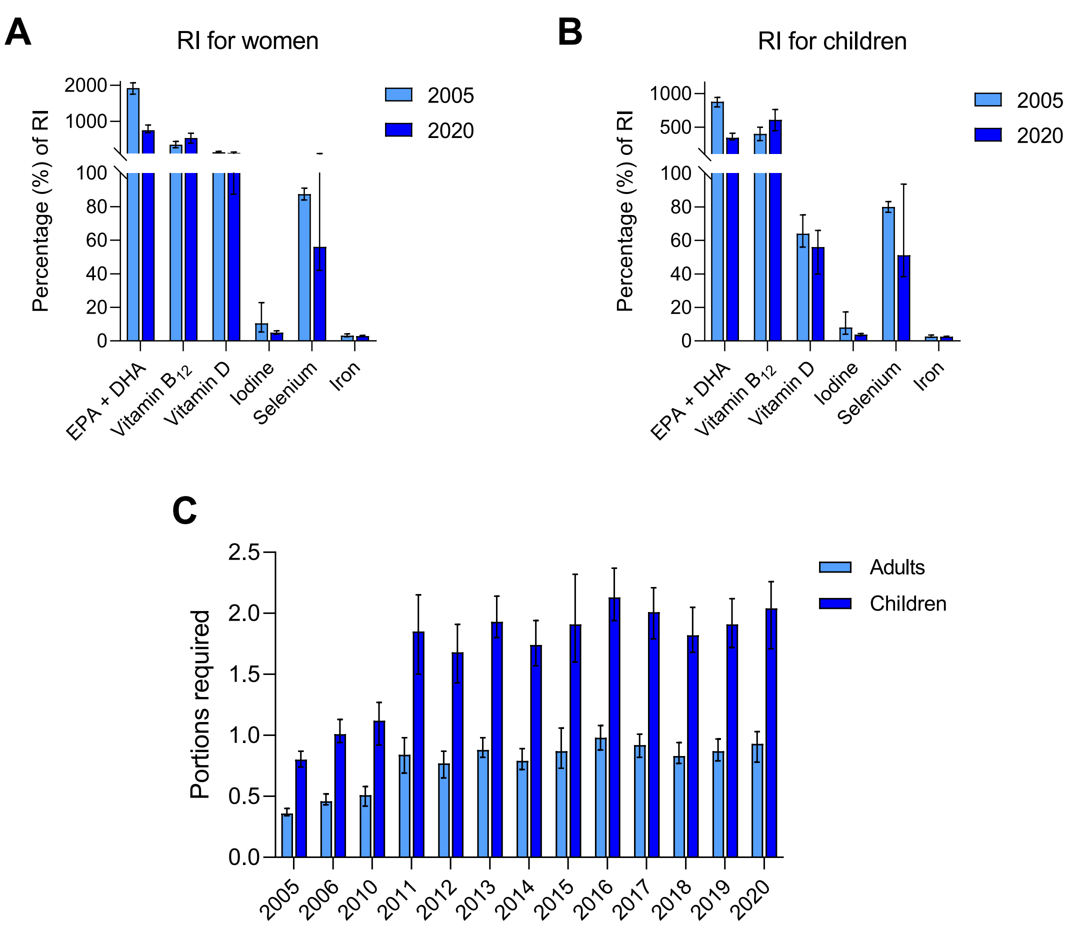

Fig. 3. The contribution of one portion of farmed Atlantic salmon (*Salmo salar*) was compared to the recommended intakes (RI) of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), vitamin B_{12}, vitamin D, iodine, selenium, and iron for (A) non-pregnant, non-lactating, healthy women of reproductive age and (B) healthy two-year old children. The calculations are based on the RI presented in the Nordic Nutrition recommendations from 2012 (Nordic Nutrition Recommendations 2012, 2014) and the adequate intake (AI) presented by the European Food Safety Authority (EFSA) (EFSA, 2010). One portion size is 175 g for women and 80 g for children. The values are based on the median nutrient content in the salmon fillets in 2005 and 2020, and the whiskers represent the interquartile range (IQR). Figure C shows the number of portions required (median and interquartile range) to obtain a weekly intake of 1.75 g EPA + DHA (250 mg/day × 7 days) based on a 175 g serving of farmed Atlantic salmon for adults and a 80 g serving for two-year old children.

for two-year-olds would be met when consuming a portion of farmed Atlantic salmon from 2005, whereas approximately 530% and 610%, respectively, would be met when consuming a portion from 2020. Furthermore, one portion of farmed Atlantic salmon was sufficient to provide 140% of the RI for vitamin D for women in 2005, whereas in 2020, one portion provided approximately 120% of the RI for vitamin D. For children, one portion from 2005 provided 64% of the RI for vitamin D, whereas one portion from 2020 was able to provide 56% of the RI. For iodine, approximately 10% of the RI would be met in 2005 for both women and children, whereas only about 5% would be met in 2020 for both groups. The RI for selenium was met by approximately 80% by women and children when a portion from 2005 was consumed, whereas a portion from 2020 was able to provide approximately 50% of the RI for both groups. The farmed salmon from both 2005 and 2020 was only able to provide approximately 3% of the RI for iron for both women and children.

4. Discussion

This study has compiled analysed contents of selected nutrients in >1100 samples of Norwegian farmed Atlantic salmon. The salmon fillets were sampled between 2005 and 2020 from various locations with aquaculture activity along the coast of Norway, thereby providing a thorough overview of the temporal changes in the nutrient content of Norwegian farmed Atlantic salmon over a time period with substantial changes in feed composition. In this study, we show that the nutrient composition of farmed Atlantic salmon has changed considerably over the past 15 years, with significantly decreasing contents of EPA + DHA and a corresponding increasing content of omega-6 fatty acids as the most prominent findings. Nevertheless, farmed Atlantic salmon remains a good source of EPA + DHA for consumers, where one and two portions are sufficient to provide the AI of EPA + DHA for a whole week for adults and two-year-olds, respectively. We also show that farmed Atlantic salmon may still be considered a good source of vitamin D_3 in the diet, as one portion sufficiently meets the daily RI of women and provides >50% of the daily RI of two-year-old children. Furthermore, one serving of farmed Atlantic salmon provides >100% of the RI of vitamin B_{12} and approximately 50% of the RI of selenium for both women and children and may therefore also be considered a considerable source of these nutrients. However, our study reveals that farmed Atlantic salmon is still a poor source of iodine and iron.

4.1. Changes in the fatty acid composition of farmed Atlantic salmon

The data presented in this study show a clear decline in the content of total omega-3 fatty acids, particularly the marine omega-3 fatty acids EPA and DHA, in Norwegian farmed Atlantic salmon from 2005 to 2011. As dietary lipid content influences the body composition of the salmon (Sissener, 2018), this is presumably a result of the decreased inclusion level of marine ingredients in salmon feeds and increased use of vegetable oils. In line with this, the observed steep decreasing slope in the contents of EPA + DHA between 2005 and 2011 corresponds in time with the concomitant reduction in use of marine ingredients in the fish feed, as reported by Ytrestøyl et al. (Ytrestøyl et al., 2015). Our data reported for Norwegian farmed Atlantic salmon are consistent with the data reported by Sprague et al. (Sprague, Dick, & Tocher, 2016) for over 3000 samples of Scottish farmed Atlantic salmon, where the contents of EPA + DHA were reported to have decreased from 2.74 g in 2006 to 1.36 g/100 g in 2015. Despite an overall declining trend for the past 15 years, the median EPA + DHA contents have remained relatively stable at 1.03–1.30 g/100 g between 2011 and 2020. Data on the composition of salmon feed from 2012 to 2016 show that the content of marine ingredients used in the feed have remained relatively stable within this time period (Aas et al., 2019). Our results further suggest that the use of marine ingredients may have remained comparatively stable in the following years as well.

Norwegian farmed Atlantic salmon still remains a very good source of EPA + DHA for human consumption, where one portion per week for adults and two portions per week for two-year-old children are sufficient to meet the EFSA's recommended intake of 250 mg/day for a whole week (EFSA, 2010). This is in line with the results presented by Sprague et al. (Sprague et al., 2016), where the authors concluded that farmed Atlantic salmon remains a considerably better source of EPA + DHA than most other fish species and all terrestrial livestock, although one would require double the portion size to meet dietary recommendations for EPA + DHA in 2015, as compared to 2006. The EPA + DHA contents in other fatty fish species, such as herring and mackerel, have previously been reported to supersede that of farmed Atlantic salmon, with 2.2 and 5.2 g/100 g, respectively (Nøstbakken et al., 2021). Nevertheless, farmed salmon is by far the most consumed fish species in Norway (Norges Sjømatråd [Norwegian Seafood Council], 2018), and thus contributes to the recommended intake of EPA + DHA to a higher degree than other fish species with a higher content of the marine n-3 LC-PUFA. According to the most recent Norwegian national dietary surveys (Astrup, Borch Myhre, Frost Andersen, & Kristiansen, 2020; Holm Totland et al., 2012), the average intake of fatty fish in the adult population is estimated to 105 g/week (15 g/day) for men and 98 g/week (14 g/day) for women with a median intake of 0 g/day for both. For two-year-old children the average intake is estimated to be 21 g/week (3 g/day; no differences between genders). By assuming farmed Atlantic salmon is the primary fatty fish species consumed, these amounts of farmed Atlantic salmon would only be able to provide 64%, 60%, and 13% of the weekly AI of EPA + DHA for men, women, and two-year-olds, respectively. Considering these data to be heavily skewed, the provision is even far less. The Norwegian health authorities recommend a weekly intake of at least 200 g fatty fish of the recommended 300–450 g total fish (Nasjonalt råd for ernæring, 2011), and as our study confirms, meeting this particular dietary recommendation is of great importance for EPA and DHA intakes, as lean fish species contain significantly less EPA and DHA than fatty fish species.

The changes in the feed composition of farmed Atlantic salmon is also reflected in the increased content of vegetable fatty acids in the salmon fillets. With the increased inclusion level of vegetable oils that have a high omega-6 fatty acid content (Sissener, 2018), our data confirm that the content of omega-6 fatty acids in the salmon fillets has significantly increased over the past 15 years. In line with this, we also observed an increased content of the characteristic vegetable fatty acids often found to be dominating in various vegetable oils, such as oleic acid (18:1n-9), LA (18:2n-6), and ALA (18:3n-3) (Sissener, 2018). This increase in the omega-3 fatty acid ALA is the main reason why we observed a higher decrease in the content of EPA + DHA (61%) compared to the decrease in the content of total n-3 fatty acids (39%) over the past 15 years. Nevertheless, farmed Atlantic salmon still contains lower levels of omega-6 fatty acids compared to many other foods, which in addition do not contain high contents of the beneficial n-3 LC-PUFA (Mattilsynet [The Norwegian Food Safety Authority], 2019).

4.2. Farmed Atlantic salmon remains a good source of vitamin D_3

As a fatty fish, salmon is often widely described as a particularly good source of vitamin D_3 (Jakobsen, Smith, Bysted, & Cashman, 2019; Schmid & Walther, 2013), however, concerns of decreasing contents of vitamin D_3 and consequently lower impact on vitamin D status when consuming farmed fish have previously been expressed (de Roos, Sneddon, Sprague, Horgan, & Brouwer, 2017; Sissener, 2018). The existing literature on vitamin D in farmed Atlantic salmon is limited, but in a review article from 2019, mean vitamin D_3 contents of 5.8–7.6 μg/100 g fillet were reported (n = 109, both pooled samples and individual samples were included) (Jakobsen et al., 2019). In this study, the median vitamin D_3 content varied from 6.0 to 9.2 μg/100 g between 2005 and 2020, thus presenting some values that exceed those already presented in the literature. Furthermore, the vitamin D_3 value of 10 μg/100 g fillet presented in the Norwegian food composition table (Mattilsynet [The Norwegian Food Safety Authority], 2019), derived from fillets sampled in 2006–2008, is slightly higher than the values reported in this study. In comparison, mean vitamin D_3 values for wild Atlantic salmon is reported to range between 9.4 and 30.0 μg/100 g (Jakobsen et al., 2019), whereas recent values from Norway indicate a content of between 10 and 11 μg/100 g fillet (Institute of Marine Research, 2020), which is not too far from the values reported for farmed Atlantic salmon in this study.

Like other vertebrates, salmons do not have any endogenous synthesis of vitamin D and are therefore dependent on dietary sources. The formulated feed of farmed Atlantic salmon allows for a more controlled supply of vitamin D_3 to the fish (Lock, Waagbø, Wendelaar Bonga, & Flik, 2010), which is a plausible explanation for why reported vitamin D_3 values for wild Atlantic salmon vary considerably more than those reported for farmed Atlantic salmon. The content of vitamin D_3 in commercial salmon feed significantly decreased from 2002 to 2008 (0.26 mg/kg (Sissener et al., 2013)) and further to 2019 (0.12 mg/kg (Ørnsrud et al., 2020)), however, a concomitant decrease in salmon fillets was not observed in this study. The vitamin D_3 content was found to have remained relatively stable, although some significant differences between years were observed. This variation may be caused by variations in the origin of the marine ingredients used in the feed, i.e. which forage fish species were utilised and the geographical origin of the fish by-products, as the origin of the marine ingredients used in the salmon feed changes according to price and availability (Ytrestøyl et al., 2015). Nevertheless, one serving of farmed Atlantic salmon would still be able to provide 100% of the RI for adults with a content as low as 6 μg/100 g fillet, which is the lowest median content reported in this study (for years 2012 and 2015). Thus, the median vitamin D_3 values from all years investigated between 2005 and 2020 suggest that one portion of farmed Atlantic salmon would be able to sufficiently meet the RI for adults by 100% or more, deeming farmed Atlantic salmon to still be considered a good source of vitamin D_3.

Vitamin D is a lipid soluble vitamin. Yet, the lack of correlation between the vitamin D_3 content and the fat content in the fillets of farmed Atlantic salmon suggests the widely accepted assumption that the content of vitamin D_3 in fish increases with the fat content may not necessarily be accurate. This observation is in line with several other studies evaluating the vitamin D_3 content in a diverse range of fish species (Aakre et al., 2020; Malesa-Ciećwierz & Usydus, 2015; Mattila, Piironen, Uusi-Rauva, & Koivistoinen, 1995; Reksten et al., 2020), as well as in wild salmon (Jakobsen et al., 2019). It has been suggested that the variation in vitamin D_3 content within fish species may be of a similar magnitude to the variation observed between fish species, and that the diet is the main contributor to this variation (Lock et al., 2010). However, in a recent study evaluating the vitamin D_3 content in 13 samples of farmed Atlantic salmon, Jakobsen et al. (Jakobsen & Smith, 2017) reported a clear linear relationship between the fat and vitamin D_3 content in the salmon fillets. Fatty fish is frequently emphasised as a superior source of vitamin D_3 compared to non-fatty fish species in dietary guidelines and recommendations (Ministeriet for Fødevarer [The Danish Veterinary and Food Administation], 2020; Nasjonalt råd for ernæring, 2011), however, our results suggest that more studies are needed to evaluate this widely accepted statement.

4.3. *Farmed Atlantic salmon is a poor dietary source of iodine*

The results of this study show that the median iodine content in farmed Atlantic salmon has varied between 2.1 and 5.4 μg/100 g between 2011 and 2020. As fish absorb iodine from surrounding seawater and the food they consume (Haldimann, Alt, Blanc, & Blondeau, 2005; Julshamn, Dahl, & Eckhoff, 2001), the significantly higher iodine content reported in 2005 (9.0 μg/100 g) is likely resulting from changes in the feed composition of farmed Atlantic salmon over the years. From 2000 to 2006, a significant declining trend in the content of iodine in salmon feed has been reported, likely attributed to the replacement of marine ingredients in fish feed (Sissener et al., 2013). However, from 2007 to 2018, the iodine content in the feed was found to be relatively stable (Sanden et al., 2013; Sele et al., 2019), with an exception for 2012 where the mean content reached a low. In this study, the lowest median iodine content was correspondingly reported in 2012 (2.1 μg/100 g), indicating that the iodine content in salmon fillets may be influenced by the iodine content in the fish feed.

Compared to other marine fish species, farmed Atlantic salmon contain a considerably lower content of iodine. In a study by Nerhus et al. (Nerhus et al., 2018) the mean iodine content was determined in several lean fish species and was in general much higher (21–790 μg/100 g) in comparison with the farmed Atlantic salmon in our study. Of available values reported for salmon, Julshamn et al. (Julshamn et al., 2001) reported a mean iodine content of 15 μg/100 g in wild Atlantic salmon in 1998, whereas in the Norwegian food composition table (Mattilsynet [The Norwegian Food Safety Authority], 2019), the reported iodine content in raw farmed Atlantic salmon is 5.5 μg/100 g. In Norway, the RI of iodine is 150 μg for adults and children older than 10 years (Nordic Nutrition Recommendations 2012, 2014), which is in line with the recommendations set by the World Health Organization (WHO) (WHO, 2001). Theoretically, if using 5 μg/100 g salmon fillet, the approximate median value for the past three years, an adult would have to consume about 17 portions of farmed Atlantic salmon to be able to meet 100% of the daily RI of iodine. In comparison, one portion of most marine lean fish species would be more than sufficient to provide 100% of the RI of iodine (Nerhus et al., 2018). As demonstrated in this study, farmed Atlantic salmon contains a quite low content of iodine and should therefore not be emphasised as a considerable source of iodine to secure an adequate iodine status.

5. Strengths and limitations

Lipid levels are known to vary throughout the flesh of the fish, with increasing concentrations from tail to head and decreasing concentrations from dorsal to ventral (Fengle et al., 2014). Thus, the inclusion of two tissues of different anatomical origin, fillets and standardised NQC, may have influenced the results. Unpublished data from the IMR on 34 individual samples of farmed Atlantic salmon from 2017, where a fillet sample was collected from one side of the fish and a NQC sample from the other, confirm that the sampling technique may affect the analytical results of some nutrients. This is not surprising, as the fillet sample encompasses a considerably larger part of the muscle tissue than the NQC, including parts of the muscle tissue where the highest content of fat is located (Fengle et al., 2014). Nevertheless, the NQC is commonly used as a representative for the quality of the salmon fillet, including the fat content (Veliyulin, van der Zwaag, Burk, & Erikson, 2005; Johnsen et al., 2011; Brown, Kube, Taylor, & Elliott, 2012), and as our statistical analyses showed, the fat contents of the fillets sampled in 2005 and 2010 were comparable to those of the NQC sampled in 2015, 2017, 2018, and 2019. Likewise, fat contents of the fillets sampled in 2006 were comparable to those of the NQC sampled in 2011 and 2012 (Table 2, Supplementary material). Furthermore, a larger part of the muscle tissue than what is encompassed in the NQC sample is available on the market and is commonly consumed, and the nutrient content available to the consumers may thus vary according to which pieces of the salmon fillets are consumed. The calculations of farmed Atlantic salmon's potential contribution to the RI of women and two-year-olds performed in this study therefore remains calculations of the potential contribution of the nutrients. Furthermore, the individual samples of farmed Atlantic salmons included in this paper were all considered to be slaughter ready, although the individual body weight at slaughter varied considerably (Table 1). Farmed salmons with a low body weight still enter the market and is available for human consumption, often at fish retailers and fish markets sold as whole fish and not fish fillets. Thus, the samples included in this paper represent a wide range of farmed salmon available to the market and for consumption.

In this paper, the portion size of two-year old children was estimated to be approximately half of that of an adult (80 g), as no standardised portion size for fish for this age group currently exists in Norway. According to the most recent national dietary survey among Norwegian two-year-olds (Astrup et al., 2020), the average intake of fish and fish products was 33 g per day in 2019, which translates to 231 g per week. By assuming the Norwegian dietary recommendations of consuming fish for dinner 2–3 times a week are followed, it can be estimated that one portion of fish for two-year-old children lies between 77 and 116 g. However, according to the dietary survey, the average intake of fish as spread/topping was 8 g per day (56 g per week). It may therefore be assumed that one dinner portion of fish for this age group is in the lower end of 77–116 g, which makes 80 g a reasonable portion size for this age group, although highly estimated. Furthermore, it should be recognised that various methods of food processing may influence the supply of nutrients available to the consumers (Greenfield & Southgate, 2003), which was not assessed in this paper where only values for raw, unprocessed salmon were used in the calculations of the potential contribution to RI. Nevertheless, the data presented in this study are of high quality, as a high number of samples collected from a wide range of fish farms over a long time-period were included. Additionally, all analyses were performed at a national reference laboratory using accredited methods. The up-to-date analytical data presented in this paper may therefore represent an important contribution to the Norwegian food composition table and future national and international risk–benefit assessments of fatty fish consumption. This is of particular importance in view of the considerable changes in the feed composition of farmed Atlantic salmon that have already taken place over the past decades and most likely will continue to evolve in the future.

6. Conclusions

In this paper, we show that the contents of marine omega-3 fatty acids, particularly EPA and DHA, have significantly decreased over the past 15 years in Norwegian farmed Atlantic salmon. However, this study also demonstrates that farmed Atlantic salmon is able to provide consumers with high amounts of EPA and DHA, with one portion for adults and two portions for two-year-old children being sufficient to meet the weekly recommended intake. We further showed that Norwegian farmed Atlantic salmon remains a good source of vitamin D_3, however, it is not a considerable source of iodine or iron. Nevertheless, farmed Atlantic salmon is overall a considerable source of several key nutrients, such as protein, EPA + DHA, vitamin B_{12}, vitamin D_3, and selenium, that may positively contribute to the nutrient intake of consumers. The updated analytical data presented in this study for fillets of farmed Atlantic salmon represent an important contribution to the Norwegian food composition table and future risk–benefit assessments on fatty fish consumption.

CRediT authorship contribution statement

Amalie Moxness Reksten: Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Quang Tri Ho:** Formal analysis, Visualization, Writing – review & editing. **Ole Jakob Nøstbakken:** Formal analysis, Writing – review & editing. **Maria Wik Markhus:** Conceptualization, Writing – review & editing. **Marian Kjellevold:** Conceptualization, Writing – review & editing. **Annbjørg Bøkevoll:** Validation, Resources, Writing – review & editing. **Rita Hannisdal:** Resources, Investigation, Writing – review & editing. **Livar Frøyland:** Conceptualization, Writing – review & editing, Funding acquisition. **Lise Madsen:** Conceptualization, Writing – review & editing, Supervision. **Lisbeth Dahl:** Conceptualization, Validation, Investigation, Resources, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

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