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FATTY ACID COMPOSITION AS INDICATOR OF FOOD INTAKE IN COD LARVAE (Gadus morhua L.) FROM LOFOTEN, NORTHERN NORWAY

by

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ABSTRACT

The year class strength of the Arcto-Norwegian cod is mainly determined during the early life stages. The mortality is caused both by starvation and predation which are linked to environmental factors during this period. Stomach content analysis of first feeding larvae show that nauplii of Calanus finmarchicus is the most important diet. The larvae are, however, frequently found with "green gut" content.

The fatty acid composition of lipids in marine animals reflects both the diets and bio-synthetic activities of the animals. The fatty acid composition of the most important prey items can be used as an indicator of the dietary lipids intake of the cod larvae.

The fatty acids of total lipid were analysed from phytoplankton, eggs and nauplii of Calanus finmarchicus and cod eggs and larvae from Lofoten waters. Gas chromatographic and gas chromatographic-mass spectrometric methods were used in the analyses.

On the basis of these analyses we suggest that the lipids of phytoplankton form an important part of the diets of cod larvae during the first feeding period and that calanoid nauplii are more important at later larval stages.

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INTRODUCTION

The most important spawning grounds of the Arcto-Norwegian cod are found in the Lofoten area, especially on the west side of the Vestfjord. The stock migrates down from the feeding areas in the Barents Sea in December and January and spawn in February, March and April. The mean peak spawning period is between March 28th and April 3rd with very little interanual variation (Pedersen, 1984).

The year class strength of the Arcto-Norwegian cod is determined during the first year (Hjort, 1914) and it is generally accepted that starvation and predation during the early stages may be important mortality factors. Recent data, however, seem to support the idea that the year class strength is strongly influenced by environmental factors. Ellertsen et al. (1986) have shown that interanual variation in sea temperature affects the time of hatching and consequently the time of cod larval start feeding which is matched or mismatched to the primary and secondary production.

Analyses of the stomach content of cod larvae from Lofoten shows that nauplii from Calanus finmarchicus is the most important item in the diet (Wiborg, 1948; Ellertsen et al., 1984). The larvae, however, are often found with "green gut" during the early start feeding period. It is not known whether this indicates direct feeding on phytoplankton or pigmented remains of gut content of digested micro zooplankton. The dietary requirements during the early larval period of cod is unknown.

The fatty acid composition of lipids in marine animals reflects both the diet and the internal bio-synthetic activities of the animals (Sargent and Falk-Petersen, 1981; Falk-Petersen, 1987). If the fatty acid composition of the most important prey items is known, specific fatty acids can be used as an indicator of the dietary lipid input to the cod larvae.

In this study, the fatty acid composition of phytoplankton, C. finmarchicus eggs and nauplii, and cod eggs and larvae was determined in order to shed some light on the dietary composition of the main larval prey organisms and larval requirements during the early start feeding period.

MATERIALS AND METHODS

Sampling

Sampling of cod eggs and larvae, mixed phytoplankton, copepod eggs and nauplii was performed in the Lofoten area during April 1985 from the research vessels F/F "G.O. Sars" and F/F "Eldjarn". Sampling positions are given in Fig. 1. Ovulated eggs were sampled from 6 cods during migration to the spawning grounds, 50 eggs in each sample. A plankton net was used to sample cod larvae. The larvae were staged according to Fossum (1986) and sorted into three groups: stage 2-4, stage 5-8 and stage 9. The samples of stage 9 larvae consisted of 1 to 3 indivuduals and the other samples of 15 to 20 individuals.

Nauplii of C. finmarchicus were sampled by a submersible electric pump (250 l/min), which pumped samples on deck through a 50 m long by 5 cm diametre hose. Samples were collected in calibrated tanks (23.7 l), and zooplankton were filtered through 90 µm mesh plankton net. The nauplii were collected with a fine glass pipette. Eggs from C. finmarchicus were obtained by putting fertile copepods into a bucket with filtrated water and, after a few hours, collecting the liberated eggs with a fine glass pipette. On board, the shaking of the water in the bucket induces spontaneous spawning. A total of 900 eggs and 1000 nauplii were sampled. These samples were taken on only one station (see Fig. 1).

Mixed phytoplankton were taken on stations 1, 2, 3, 5 and 6 (see Fig. 1) with a 5 l Niskin sampler and collected on Whatman GF/C glass fibre filters. Macro-zooplankton were first removed by filtering the water through 90 µm pads.

All samples for fatty acid analyses were stored at ÷20°C in a solution of 0.05% butylated hydroxytoluene (BHT) in chloroform: methanol (2:1/v:v) before they were analysed. Phytoplankton samples for total carbon measurements were stored at ÷20°C in small glass tubes. Samples for determination of phytoplankton composition were put in 100 ml bottles, conserved with 4% formaline and stored at room temperature.

Analysis

The solvents used were glass distilled and all glassware was rinsed in solvents and heated at 400°C for several hours prior to use. Butylated hydroxytoluene (BHT), with a concentration of 0.05%, was added to the solvents to avoid oxidation of the fatty acids during the experiment. Complete procedural blanks were run with every group of samples.

Lipid extraction was done using the method described by Folch et al. (1957). Samples were ground up in a chloroform:methanol (2:1/v:v) mixture using a Potter Elvehjem homogenizer. Methyl esters of fatty acids from the total lipid extracts were prepared using H_2SO_4-catalyzed methylation in dry methanol (Christie, 1982). The methyl esters were recovered by hexane extraction after addition of 5% NaCl solution. Aliquots of the methyl ester extracts were purified using 250 µm thick silica gel TLC-plates (20x20 cm) developed in hexane:diethyl ether:acetic acid, in the proportions 90:10:1 (v:v:v). Standards were simultaneously chromatographed and stained with 2',7'-dichlorofluorescein to reveal the position of the fatty acid methyl esters. The methyl esters were eluted with diethyl ether and the ether was then replaced with hexane.

The purified extracts of fatty acid methyl esters were analyzed by gas-liquid chromatography on a Hewlett Packard model 5890 equipped with a flame ionization detector and a fused silica capillary column, 30 m x 0.32 mm ID, coated with 0.25 µm DB-225. Gas chromatographic conditions were: injector temperature, 280°C; detector temperature, 250°C; column temperature, 60°C (1 min) to

160°C at 15°C/min, 160°C to 210°C at 2°C/min, 20 min at 210°C; carrier gas, H_2 at 40 cm/sec. The samples were injected by splitless injection. Individual fatty acids were identified by comparison with known standards. Further proof of identity was obtained by analysing samples by electron impact (70 eV) and methane chemical ionization on a Hewlett Packard model 5987 A gas chromatograph-mass spectrometer. The chromatographic conditions were the same as in the gas chromatographic analysis. Mass spectrometric conditions with methane chemical ionization were: transfer line, 210°C; ion source temperature, 150°C; source pressure, 400 mTorr CH_4; electron energy, 100 eV.

The quantifications were performed by external standards containing 20 of the analysed fatty acid methyl esters. Compounds not available were assumed to have similar gas chromatographic responses to those shown by the other structurally - related reference compounds.

RESULTS

Phytoplankton

The most dominant phytoplankters in the samples were phytoflagellates with *Phaeocystis pouchetii* as the most numerous. Only the main phytoplankton families are listed in Table 1. The fatty acid analysis were made on mixed phytoplankton samples and the fatty acids of the phytoplankton lipids were very rich in the short chain saturates (approx. 50%), especially 14:0 (10 to 17%) and 16:0 (20 to 26%) (Table 2). Moderate amounts of polyunsaturated fatty acids (PUFA) were also present. The PUFAs were present mainly as 20:5 (n-3) and 22:6 (n-3). Substantial levels of 18:2 (n-6) were also recorded, accounting for 6 to 9% of the total fatty acids.

Copepods

The eggs of *C. finmarchicus* were sampled from mature females which spawned spontaneously in small glass aquaria on board the

research vessel. The nauplii, however, were collected from sea water samples and 91% of the nauplii were C. finmarchicus (n=221). These nauplii were staged and 68% was in nauplii stage 1 to 3 and 32% in stage 4 to 6. Nauplii stage 3 was the most dominant and constituted 58%. At this stage the nauplii start to feed phytoplankton.

Eggs and nauplii from C. finmarchicus have high levels of PUFAs (46 and 50% respectively), especially 20:5 (n-3) and 22:6 (n-3) in their lipids (Table 3). After hatching, the (n-3)/(n-6) level increases from 11.2 to 19.2, mainly due to an increase in 22:6 (n-3) from 18.2 to 25.2%. The 20:1 and 22:1 moieties, so abundant in the copepodite and adult stage of calanoid copepods, were only minor constituents of the lipids of eggs and nauplii of C. finmarchicus.

Cod

The cod larvae were staged according to Fossum (1986) from stage 2 to 9. Larvae in stage 2 to 4 do not feed and get all their dietary requirements from the yolk. At stage 5 they start exogenous feeding. The larvae in stage 2 to 4 and 5 to 8 from the different stations were pooled respectively, and the amount of fatty acids in µg per larvae is presented in Table 4. The highest variation was found in stage 9 larvae which varied between 17 µg to 74 µg per larvae.

There is an increase in the amount of free fatty acid (FFA) from stage 2-4 to stage 9 (Table 4). From stage 2-4 to stage 5-8 there is a moderate increase in the FFA level from ca. 6 µg to 9 µg per larvae while there is a marked increase to ca. 50 µg per larvae in stage 9. This indicate that large amounts of lipids are laid down between stage 5-8 and 9.

The fatty acid composition of cod egg and larvae lipids showed little variation from egg to stage 5-8 cod larvae. The PUFA level was high and accounted for approx. 50% during this period - decreasing to 40% in stage 9. Between stage 5-8 and 9 there was

a decrease in the 20:5 (15 to 10.8%) and 22:6 (30.2 to 19.9%) PUFAs together with the 20:1 (2.6 to 1.3%) and 22:1 (0.3 to 0%) moieties. The (n-3)/(n-6) ratio decreased from 13.7 in eggs and early larval stages to 4.7 in stage 9, mainly due to an increase in 18:2 (n-6) and decrease in 20:5 (n-3) and 22:6 (n-3).

Between stage 5-8 and 9, there was a marked increase in the saturated fatty acid 14:0 (2.6 to 4.2%), 16:0 (17.4 to 20.6%) and 18:0 (5.2 to 8.6%), together with the increase in the 18:2 (n-6) from 1.7 to 6.3%. All of the fatty acids which were found to increase between stage 5-8 and 9 are also very abundant in the mixed phytoplankton.

DISCUSSION

The cod larval growth rate during early start feeding in enclosed ponds highly exceeds larval growth rate in the field, even when comparing larvae from a fjord where the larval feeding incidence is 100% and the concentration of nauplii is higher than in the enclosed pond (see Kvenseth and Øiestad, 1984 and Ellertsen et al., 1986). It is assumed that a cod larval population during start feeding meet metabolic requirement when the nauplii concentration exceeds 10-20 nauplii l^{-1} (Solberg and Tilseth, 1984; Ellertsen et al., 1986). It is not known, however, if the larvae meet its dietary requirements by feeding exclusively on nauplii.

The cod larvae develop functional jaw in stage 5, and ingestion of exogenous food starts between stage 5 and 8 (Ellertsen et al., 1981, 1984). The main prey of the cod larvae during this period are particles within the size range of 140-520 µm (Tilseth and Ellertsen, 1984; Wiborg, 1948). In experiments in an enclosed pond, Kvenseth and Øiestad (1984) found that the gut of the cod larvae were filled with a green mass and that calanoid nauplii were common in the diet after day 9 (approx. stage 7-8. See Fossum, 1986). Larvae with green gut have also been observed by the authors in the Lofoten area during the early start feeding period, as well as during a number of field studies on this

species (Barnbridge and McKay, 1948; Nordeng and Bratland, 1971; Wiborg, 1948).

As most small marine fish larvae, the cod larvae are visual feeders and size selective (Ellertsen et al., 1977, 1981). During the onset of feeding they feed on the most numerous particles within the preferred size range (Ellertsen et al., 1977). The possibility exists that during this period the larvae select immobile or slow moving particles as rotifers, one-celled phytoplankters (Nordeng and Bratland, 1971) or copepod fecal pellets. It is consistent both in gut content analyses from pond experiments (Kvenseth and Øiestad, 1984) and in larvae from the field (Ellertsen et al., 1977) that a high percentage of the larval gut content consists of pigmented unidentified material. The larval feeding success, however, increases rapidly with time (Solberg and Tilseth, 1984) and then become more efficient and probably more prey selective. This might explain the increase in numbers of copepod nauplii in the larval gut with time.

The lack of success with start feeding of cod larvae on both artificial food and copepod nauplii in intensive rearing units (Huse et al., 1984), contrary to the success in enclosed ponds, suggested that the food in intensive systems did not meet the dietary requirements of the cod larvae. The initial feeding on a variety of prey organisms in pond systems might contribute to important dietary elements.

Cod eggs have a total lipid content of 10-15% of which polar lipids account for ca. 70%. The same are found in eggs from a number of other marine species, i.e. herring, haddock, whiting, saithe and halibut (Tocher and Sargent, 1984; Falk-Petersen et al., 1986). The polar lipids in all the species contains ca 50% PUFA. This indicates that PUFA plays an essential role in the development of the fish larvae. High levels of polar lipid and PUFA are also found in the egg and nauplii stages of calanoid copepods (Sargent and Hendersen, 1986). The total lipid of the cod eggs from Lofoten contained 23.5% saturated fatty acids, 26.9% monounsaturates and 49.7% PUFA (Table 5). The composition

of individual fatty acids were much the same as Tocher and Sargent (1984) detected in the polar lipids of ripe roes of cod.

In Lofoten larvae have a slow, but continous growth from stage 5. The growth rate first seems to increase markedly between stage 8 and 9 (Ellertsen et al., 1986). This is also in agreement with our findings, where the lipid level in larvae (as µg FFA) increased rapidly in the same period (Table 4). The same pattern can be seen in the fatty acid composition. There was little change in this from eggs to stage 5-8 larvae. However, in stage 9 there was an increase in 14:0, 16:0, 18:0, 18:2 (n-6) and a decrease in 20:5 (n-3), 22:6 (n-3), 20:1 (n-9) and 22:1 (n-11). All the fatty acids which increased in stage 9 were the same as those found in the phytoplankton lipid where 18:2 (n-6) can be used as the most specific marker (Table 2).

The phytoplankton species composition in the area indicate that the characteristic spring bloom had already taken place. Although numerically the phytoflagellate Phaeocystis pouchetii was the most dominant species, due to its small size (4-5 µm in diameter), it does not contribute greatly to the total phytoplankton biomass. On the other hand, the more moderate numbers of different types of diatoms of a much larger size makes the main bulk of the phytoplankton biomass. The relatively high numbers of resting spores of Chaetoceros socialis and C. furcellatus suggest that the main diatom spring bloom was over.

There was little indication of any incorporation of specific fatty acids of nauplii origin in the cod larvae. On the basis of these results we conclude that the lipids of phytoplankton origin may be an important part of the diet of cod larvae during the initial part of the first feeding period. This is consistent with the observation that the larvae feed on a variety of microzooplankton-particles rich in pigmented material, which most probably are of phytoplanktonic origin.

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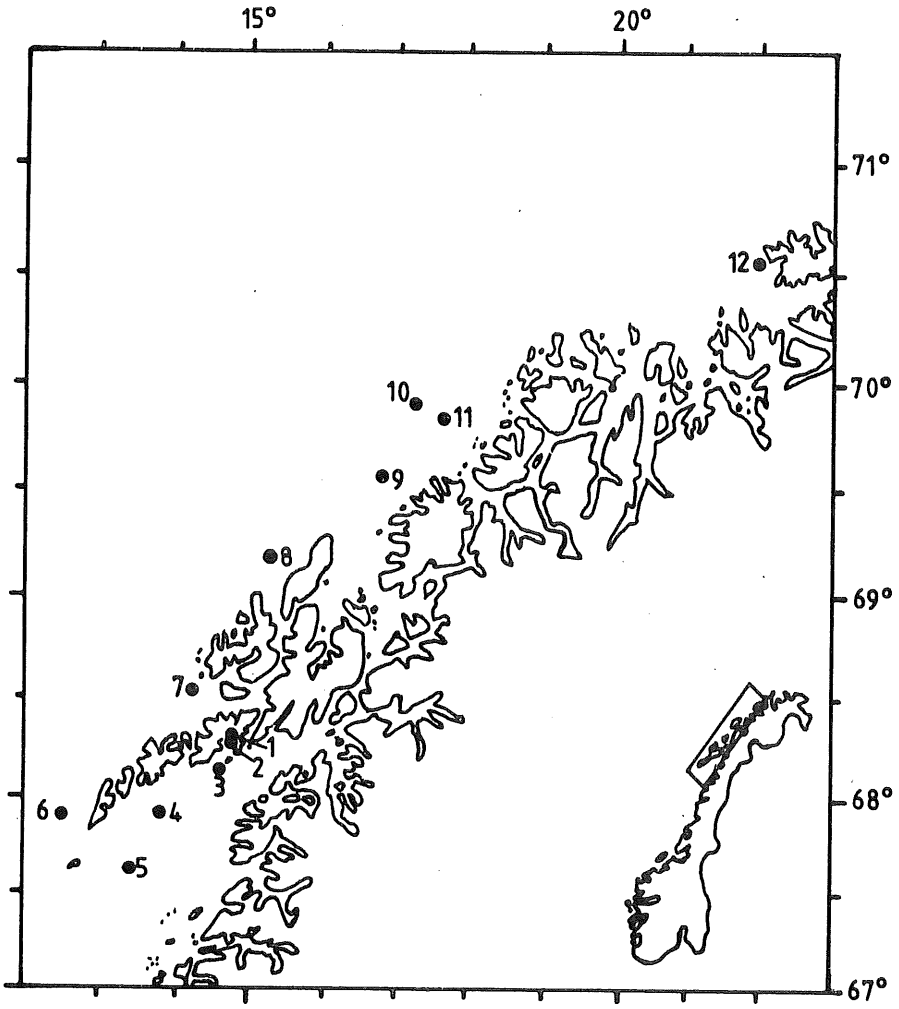

Fig. 1. Sampling stations in Lofoten-Vesterålen area in 1985. Phytoplankton were collected at station 1, 2, 3, 5 and 6, cod larvae at station 1, 3, 4, 6 and 7, copepods eggs and nauplii on station 7 and mature female cod were trawled and the eggs stripped on station 8 to 11.

Table 1. Phytoplankton composition (no. x 10^6/1) and concentrations (µg/1) of particulate organic carbon (POC), chlorophyll A (Chl. A) and fatty acids (FA). The samples were taken between April 22 and April 26 1985. (Station no. see Fig. 1.)

	Station				
	1	2	3	5	6
Dinophyceae sp.	0.07		0.3	0.04	0.07
Prymnesiophyceae sp.	3.7		4.5	2.1	2.3
Bacillariophyceae sp.	0.8		0.2	0.3	0.1
Undetermined flagellates/ monades sp.	6.0		2.8	2.0	1.1
POC (µg/1)	438	204	276	243	150
Chl. A (µg/1)	3.63	-	3.84	3.96	1.65
Total FA (µg/1)	65	29	26	34	18

Table 2. Fatty acid composition of total lipid from mixed phytoplankton. The stations are shown in Fig. 1.

	Station 1	2	3	5	6
14:0	17.4	13.7	12.0	10.8	10.2
15:0	1.3	1.1	1.4	2.2	1.9
16:0	24.8	20.3	24.7	24.2	25.7
16:1(n-9)	1.7	2.7	2.4	3.7	3.2
16:1(n-7)	6.9	6.4	10.5	4.2	3.2
16:2(n-4)	0.5	0.7	0.9	0.6	0.5
16:4(n-3)	0.7	1.2	1.0	1.3	1.3
17:0	0.7	0.8	1.8	1.3	1.9
18:0	8.4	9.3	7.2	11.1	12.6
18:1(n-9)	6.7	8.9	8.0	13.6	13.0
18:1(n-7)	1.1	1.1	2.2	1.5	1.7
18:1(n-5)	0.3	0.7	0.1	0.3	0.3
18:2(n-6)	7.6	9.0	6.5	6.4	6.1
18:3(n-6)	0.2	0.2	0.3	0.1	-
18:3(n-3)	1.4	2.0	3.2	1.9	2.5
18:4(n-3)	4.8	6.2	5.9	4.2	4.5
20:0	1.0	0.8	0.6	1.0	1.2
20:1(n-9)	0.1	1.0	-	1.3	1.0
20:1(n-7)	-	-	-	-	-
20:4(n-6)	0.4	0.2	0.2	0.1	-
20:4(n-3)	-	0.2	0.2	0.2	0.2
20:5(n-3)	4.7	5.3	5.8	5.1	4.4
22:0	0.4	0.4	0.3	0.6	0.6
22:1(n-11)	0.2	-	-	-	0.3
22:1(n-9)	-	-	-	-	-
22:5(n-3)	1.2	0.5	0.2	0.4	0.5
22:6(n-3)	7.5	7.5	4.4	4.1	3.4
24:1(n-9)	-	-	-	-	-
Total saturates	53.9	46.3	48.0	51.2	54.2
Total mono-unsatuates	17.0	20.8	23.3	24.6	22.7
Total (n-3)	20.3	22.9	20.7	17.2	16.8
Total (n-6)	8.2	9.4	7.0	7.5	6.1
(n-3)/(n-6)	2.5	2.4	3.0	2.4	2.8

Table 3. Fatty acid composition of total lipid of eggs and nauplii from Calanus finmarchicus.

	Copepod eggs	Copepod nauplii
14:0	4.0	4.1
15:0	0.8	1.2
16:0	21.2	19.4
16:1(n-9)	0.9	1.4
16:1(n-7)	4.8	3.0
16:2(n-4)	0.5	0.4
16:4(n-3)	0.4	0.2
17:0	0.5	0.7
18:0	3.7	4.3
18:1(n-9)	6.9	5.3
18:1(n-7)	3.0	2.1
18:1(n-5)	0.8	0.6
18:2(n-6)	2.3	1.6
18:3(n-6)	0.3	0.2
18:3(n-3)	1.7	1.5
18:4(n-3)	2.8	2.2
20:0	0.2	0.4
20:1(n-9)	3.7	3.5
20:1(n-7)	0.2	0.2
20:4(n-6)	1.2	0.7
20:4(n-3)	0.6	0.4
20:5(n-3)	17.2	18.1
22:0	-	0.3
22:1(n-11)	1.3	0.4
22:1(n-9)	0.1	0.1
22:5(n-3)	1.1	0.5
22:6(n-3)	18.6	25.2
24:1(n-9)	1.0	1.8
Total saturates	30.4	30.4
Total monounsaturates	22.6	18.6
Total (n-3)	42.4	48.0
Total (n-6)	3.8	2.5
(n-3)/(n-6)	11.2	19.2

Table 4. Cod larvae from Lofoten - sample station, sampling date, larval stage, average amount of fatty acids per larvae.

Sample station	Sampling date	Larval stage	Amount of FA (µg)
1	22.04.84	2-4	6.3
3	01.05.85	2-4	6.9
1	22.04.85	5-8	7.7
4	26.04.85	5-8	8.3
3	01.05.85	5-8	12.1
1	22.04.85	9	17.0
6	29.04.85	9	74.0
7	30.04.85	9	50.0

Table 5. Fatty acid composition of total lipid of cod eggs and larvae.

	Cod eggs (n=6)[B]	Cod larvae 2-4[A] (n=2)[B]	Cod larvae 5-8[A] (n=3)[B]	Cod larvae 9[A] (n=3)[B]
14:0	2.4 ± 0.5	2.2	2.6 ± 0.2	4.2 ± 0.7
15:0	0.4 ± 0.08	0.5	0.6 ± 0.2	1.9 ± 0.9
16:0	18.5 ± 1.1	19.2	17.4 ± 1.2	20.6 ± 0.5
16:1 (n-9)	1.3 ± 0.2	1.6	1.4 ± 0.1	3.9 ± 1.1
16:1 (n-7)	3.2 ± 0.3	2.1	2.8 ± 1.8	1.7 ± 0.3
16:2 (n-4)	0.1 ± 0.00	0.1	0.1 ± 0.1	0.1 ± 0.06
16:4 (n-3)	0.3 ± 0.09	0.6	0.5 ± 0.2	0.4 ± 0.2
17:0	0.2 ± 0.05	0.3	0.3 ± 0.06	1.0 ± 0.6
18:0	2.0 ± 0.2	5.8	5.2 ± 0.5	8.6 ± 0.7
18:1 (n-9)	11.5 ± 1.0	9.3	7.6 ± 0.8	9.7 ± 0.6
18:1 (n-7)	4.5 ± 0.8	3.5	3.0 ± 0.3	2.1 ± 0.3
18:1 (n-5)	0.5 ± 0.08	0.6	0.5 ± 0.06	0.5 ± 0.2
18:2 (n-6)	1.1 ± 0.1	1.2	1.7 ± 0.4	6.3 ± 2.7
18:3 (n-6)	0.1 ± 0.00	0.1	0.1 ± 0.00	0.1 ± 0.00
18:3 (n-3)	0.3 ± 0.04	0.3	0.6 ± 0.00	1.1 ± 0.4
18:4 (n-3)	0.5 ± 0.2	0.3	1.4 ± 0.70	0.9 ± 0.1
20:0	-	0.3	0.2 ± 0.00	1.0 ± 0.5
20:1 (n-9)	4.0 ± 1.1	3.4	2.5 ± 1.36	1.3 ± 0.8
20:1 (n-7)	0.2 ± 0.04	0.1	0.1 ± 0.06	-
20:4 (n-6)	1.7 ± 0.4	2.4	1.8 ± 0.3	0.8 ± 0.5
20:4 (n-3)	0.4 ± 0.05	0.3	0.4 ± 0.2	0.3 ± 0.06
20:5 (n-3)	14.8 ± 1.2	13.9	15.0 ± 1.6	10.8 ± 0.8
22:0	-	0.1	0.1 ± 0.00	0.2 ± 0.06
22:1 (n-11)	0.8 ± 0.2	0.5	0.2 ± 0.00	-
22:1 (n-9)	0.1 ± 0.00	-	0.1 ± 0.1	-
22:5 (n-3)	1.2 ± 0.2	1.5	1.2 ± 0.06	0.7 ± 0.2
22:6 (n-3)	29.3 ± 1.1	28.6	30.2 ± 4.0	19.9 ± 1.7
24:1 (n-9)	0.8 ± 0.1	0.8	0.7 ± 0.06	0.9 ± 0.06
Total saturates	23.5	28.4	26.4	37.5
Total mono-saturates	26.9	21.9	18.2	20.1
Total (n-3)	46.8	45.5	49.3	33.8
Total (n-6)	2.9	3.7	3.6	7.2
(n-3)/(n-6)	16.1	12.3	13.7	4.7