NRC Publications Archive Archives des publications du CNRC

Lipid and fatty acid compositions of cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and halibut (*Hippoglossus hippoglossus*)
Duan, Zeng; Kangsen, Mai; Qinghui, Ai; Milley, Joyce; Lall, Santosh P.

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. / La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version acceptée du manuscrit ou la version de l'éditeur.

For the publisher's version, please access the DOI link below. / Pour consulter la version de l'éditeur, utilisez le lien DOI ci-dessous.

Publisher's version / Version de l'éditeur:

<https://doi.org/10.1007/s11802-010-1763-4>

Journal of Ocean University of China (English Edition), 9, 4, pp. 381-388, 2010-01-01

NRC Publications Record / Notice d'Archives des publications de CNRC:

<https://nrc-publications.canada.ca/eng/view/object/?id=285d7332-58ae-4199-a8c1-8a13fc340829>

<https://publications-cnrc.canada.ca/fra/voir/objet/?id=285d7332-58ae-4199-a8c1-8a13fc340829>

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at

<https://nrc-publications.canada.ca/eng/copyright>

READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site

<https://publications-cnrc.canada.ca/fra/droits>

LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at

PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.

Lipid and Fatty Acid Compositions of Cod (*Gadus morhua*), Haddock (*Melanogrammus aeglefinus*) and Halibut (*Hippoglossus hippoglossus*)

ZENG Duan¹⁾, MAI Kangsen^{1), *}, AI Qinghui¹⁾, Joyce E. Milley²⁾, and Santosh P. Lal²⁾

1) The Key Laboratory of Mariculture, Ministry of Education of China, Ocean University of China, Qingdao 266003, P. R. China

2) National Research Council of Canada, Institute for Marine Biosciences, 1411 Oxford Street, Halifax, Nova Scotia B3H 3Z1, Canada

(Received April 20, 2010; revised May 6, 2010; accepted June 17, 2010)

© Ocean University of China, Science Press and Springer-Verlag Berlin Heidelberg 2010

Abstract This study was conducted to compare lipid and fatty acid composition of cod, haddock and halibut. Three groups of cod (276 g±61 g), haddock (538 g±83 g) and halibut (3704 g±221 g) were maintained with commercial feeds mainly based on fish meal and marine fish oil for 12 weeks prior to sampling. The fatty acid compositions of muscle and liver were determined by GC/FID after derivatization of extracted lipids into fatty acid methyl esters (FAME). Lipids were also fractionated into neutral and polar lipids using Waters silica Sep-Pak[®]. The phospholipid fraction was further separated by high-performance thin-layer chromatography (HPTLC) and the FAME profile was obtained. Results of the present study showed that cod and haddock were lean fish and their total muscle lipid contents were 0.8% and 0.7%, respectively, with phospholipid constituting 83.6% and 87.5% of the total muscle lipid, respectively. Halibut was a medium-fat fish and its muscle lipid content was 8%, with 84% of the total muscle lipid being neutral lipid. Total liver lipid contents of cod, haddock and halibut were 36.9%, 67.2% and 30.7%, respectively, of which the neutral lipids accounted for the major fraction (88.1%–97.1%). Polyunsaturated fatty acids were the most abundant in cod and haddock muscle neutral lipid. Monounsaturated fatty acid level was the highest in halibut muscle neutral lipid. Fatty acid compositions of phospholipid were relatively constant. In summary, the liver of cod and haddock as lean fish was the main lipid reserve organ, and structural phospholipid is the major lipid form in flesh. However, as a medium-fat fish, halibut stored lipid in both their liver and muscle.

Key words cod; haddock; halibut; lipid; fatty acids; neutral lipid; phospholipid; liver; muscle

1 Introduction

The coldwater marine fish, Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and halibut (*Hippoglossus hippoglossus*), have a long history for use as human food in North America and Europe. Their importance as a source of n-3 polyunsaturated fatty acids (*i.e.* eicosapentaenoic or EPA, 20:5 n-3; docosahexaenoic or DHA, 22:6 n-3), fat soluble vitamins and high quality protein is widely recognized in human health. Currently there is much interest in the development of marine fish aquaculture in Atlantic Canada and Europe because of the declined catches from wild fisheries. As a well-established market existing for these food fish, farming could fill this gap between supply and demand and supply high quality product for human consumption. The nutritional requirements for these fish have not yet been properly defined but need to be addressed since feed accounts for a

major cost of fish aquaculture production. Lipids are considered the key component of marine fish diets because they supply both energy and essential fatty acids. The lipid composition of wild cod, haddock and halibut has been investigated (Ackman, 1989), and is known to vary according to lipid composition of preys. It can also be influenced by other factors including physiological status (*e.g.* age, sexual maturation, feeding and fasting state), activity level, size, genetics, nutrient composition and energy content of the diets, seasonal change and area of capture. Especially in cold and temperate climates, the lipid and fatty acid composition also fluctuates widely in relation to seasonal cycles.

The present study was designed to compare the lipid composition of cod, haddock and halibut fed diets of similar lipid supplements from fish oil, with an emphasis on examining the fatty acid composition of phosphoglycerides in the liver and muscle. This information would be useful for future studies on the alternative lipid sources to fish oil and how they may affect phosphoglyceride species of haddock, cod and halibut, including the effect of phospholipid PUFA on eicosanoid metabolism in these

* Corresponding author. Tel.: 0086-532-82032038
E-mail: kmai@ouc.edu.cn

fish. As the rapidly expanding aquaculture industry continues to thrive, this information will be essential to further the development of these new marine fish species.

2 Materials and Methods

2.1 Animals and Sampling

One-year-old Atlantic cod, *G. morhua*, with an average live weight of 276 g±61 g, one-year-old haddock, *M. aeglefinus*, with an average weight of 538 g±83 g, and three-year-old Atlantic halibut, *H. hippoglossus*, with an average live weight of 3704 g±221 g, were obtained from the National Research Council Aquaculture Research Station at Sandy Cove, Nova Scotia, Canada in September 2005. The fish were fed a commercial cod/haddock diet or halibut diet (Skretting, Canada) given in Table 1, and fasted for 24 h prior to weighing and counting. All the fish

were sacrificed with an overdose of TMS (tricaine methanesulfonate) and dissected. The fish liver and fillet were stored at -80°C for subsequent proximate and biochemical analyses.

2.2 Proximate and Biochemical Analyses

2.2.1 Diets

The proximate composition, energy content and fatty acid profile of fish diets were shown in Table 1. The nutritional composition of diets was analyzed according to AOAC (1990). Crude protein (%N×6.25) was measured by the Dumas methods (Ebling, 1968) using a Leco Nitrogen Determinator (Model FP-228, Leco Corporation, St. Joseph, MI, USA). Lipid was measured according to Folch *et al.* (1957). Energy content was determined using an adiabatic bomb calorimeter (model 1261, Parr Instrument Company, Moline, IL, USA).

Table 1 Proximate composition, energy content and fatty acid profile of the experimental diets^{1,2}

Component	Cod/haddock diet	Halibut diet	Component	Cod/haddock diet	Halibut diet
Lipid (%)	14.7	15.6	ΣMUFA ⁴	29.3	34.3
Crude protein (%)	49.6	49.6	18:2n-6	15.2	5.3
Gross energy (MJ kg ⁻¹ DM)	20.5	21.2	20:4n-6	0.8	1.1
Neutral lipid (%)	10.7	12.1	Σn-6 ⁵	16.6	7.3
Polar Lipid (%)	1.9	1.6	18:3n-3	2.9	1.1
14:0	4.1	5.5	18:4n-3	0.1	0.2
16:0	15.7	15.5	20:4n-3	0.4	0.4
18:0	3.7	3.3	20:5n-3	9.6	12.2
ΣSAT ³	24.8	25.5	22:5n-3	1.2	1.6
16:1n-7	5.0	6.9	22:6n-3	8.7	9.4
18:1n-9	17.9	12.7	Σn-3 ⁶	23.3	25.7
18:1n-7	3.3	2.7	ΣPUFA ⁷	44.2	38.9
20:1n-9	1.0	4.4	DHA/EPA	0.9	0.8
22:1n-11	0.9	5.7			

Notes: ¹ Values are means of two measurements. ² Data are expressed as area percentage of FAME. ³ Including 8:0, 10:0, 12:0, 13:0, 15:0, iso16:0, 7Me16:0, 17:0, 19:0, 20:0, 21:0, 22:0, 23:0 and 24:0. ⁴ Including 14:1n-5, 14:1n-7, 15:1n-5, 16:1n-5, 17:1, 18:1n-5, 22:1n-9, 22:1n-7 and 24:1n-9. ⁵ Including 16:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 22:2n-6, and 22:5n-6. ⁶ Including 16:3n-3, 16:4n-3, 20:3n-3, 21:5n-3 and 22:4n-3. ⁷ Including 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-4, 18:2n-9, 18:3n-4, 18:4n-1, 20:2n-7 and 20:2n-9.

2.2.2 Lipid extract and fatty acid analysis of fish

Lipids from fish muscle were extracted using the method of Bligh and Dyer (1959). Lipids from fish liver were extracted using the method of Folch *et al.* (1957). Fatty acid compositions of the tissue samples were estimated from the fatty acid methyl ester (FAME) derivatives of the transesterified lipids. The FAMES were prepared using 7% boron trifluoride in methanol, heated to and maintained at 100°C for 1 h (Ackman, 1998). The FAMES were then separated by a gas chromatograph (GC) equipped with a flame-ionization detector (Hewlett Packard 6890 GC system, Wilmington, DE, USA) on an Omegawax 320 capillary column (30 m×0.32 mm i.d.×0.25 μm film thickness; Supelco, Bellefonte, PA, USA). FAMES were identified by comparison of retention times with those of known standards (Supelco 37, Menhaden Oil; Supelco).

Lipids from fish muscle/liver were separated into neutral lipid and polar lipid according to the procedures de-

scribed by Juaneda and Rocquelin (1985). The total lipid extracts (100–200 mg) were fractionated on silica cartridges (Sep-Pak[®] Vac 6cc, Waters); neutral lipid was eluted with chloroform and polar lipid with methanol. Fatty acid compositions of neutral lipid and polar lipid were determined with the same procedures as those for the total lipids.

2.2.3 Phospholipid analysis of fish

Phospholipid from cod and halibut muscle/liver was separated to determine lipid class by high-performance thin-layer chromatography (HPTLC) (Vitiello and Zannetta, 1978; Olsen and Henderson, 1989). Sixty plates (10×10 cm) of pre-coated HPTLC silica gel plates were obtained from E. Merck (Darmstadt, Germany). Lipid standards (phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine) were obtained from Doosan Serdary Re-

search Laboratories (Toronto, ON, Canada).

The HPTLC plates were pre-developed to full length in hexane: diethyl ether (1:1) to remove impurities. After drying under a stream of hot air, 300 µg–400 µg of lipid sample was added onto the concentration zone area of HPTLC plate using a glass micro-syringe. A standard lipid mixture, including phosphatidic acid, phosphatidylcholine, phosphatidylethanoamine, phosphatidylinositol and phosphatidylserine, in chloroform was spotted in each HPTLC plate to indicate the different lipid class bands.

The plates were developed using methyl acetate: isopropanol: chloroform: methanol: 0.25% KCl (25:25:25:10:9) as the solvent system until the solvent front reached 1 cm from the plates' top.

Lipid class standards were detected by spraying the plates with 0.1% 2',7'-dichlorofluorescein solution and visualized under UV light. The adjacent band for the sample was scraped individually. Extracted lipid by the Vitiello and Zanetta (1978) method from the scraped band was determined via FAME as described above.

2.2.4 Cholesterol analysis

About 50 mg of lipid extract was saponified with 0.5 mL 50% KOH and 2 mL 95% ethanol following the method of Kovacs (1979). After boiling for 1 h and cooling, 1.5 mL of distilled water was added and the unsaponifiables were extracted with hexane for 4 times (2.5 mL each). The combined extracts were concentrated and analyzed for

free sterols using GC equipped with a flame-ionization detector (Hewlett Packard 6890 GC system, Wilmington, DE, USA) on SAC-5 capillary column (30 m×250 mm i.d.×0.25 µm film thickness; Supelco, Bellefonte, PA, USA).

2.3 Statistical Analysis

All data were presented as mean±SD (n=5). Data were subject to one-way analysis of variance by IBM SPSS version 10.0. Statistical significance was determined at $P < 0.05$ for each set of comparisons.

3 Results

3.1 Hepatosomatic Index (HSI), Lipid Content of Fish Muscle and Liver

Hepatosomatic index (HSI) = $100 \times \text{wet liver weight} / \text{body weight}$

HSI of haddock (7.0) was much higher than that of cod and halibut (2.4 and 2.0, respectively) (Table 2). Haddock liver also contained much more lipid (67.2%) than the other two fish species (36.9% and 30.7%, respectively). Halibut muscle contained approximately 10 times more lipids (8.0%) than cod and haddock muscle (0.8% and 0.7%, respectively). Neutral lipid was the dominant lipid class in all fish livers as well as in halibut muscle. In contrast, the major lipid class in cod and haddock muscle was polar lipid.

Table 2 Hepatosomatic index (HSI), lipid and cholesterol content of cod, haddock and halibut¹

Component	Cod	Haddock	Halibut
HSI ²	2.4±1.0 ^a	7.0±2.1 ^b	2.0±0.2 ^a
Muscle lipid (%)	0.8±0.1 ^a	0.7±0.1 ^a	8.0±4.0 ^b
Liver lipid (%)	36.9±7.3 ^a	67.2±3.5 ^b	30.7±4.8 ^a
Muscle neutral lipid (%)	0.11±0.03 ^a	0.09±0.1 ^a	6.9±4.3 ^b
Muscle polar lipid (%)	0.64±0.04 ^a	0.61±0.1 ^a	1.3±0.04 ^b
Liver neutral lipid (%)	33.5±7.5 ^a	65.2±3.0 ^b	27.1±5.2 ^a
Liver polar lipid (%)	2.4±0.6 ^a	1.4±0.4 ^a	3.1±1.3 ^a
Muscle cholesterol content (mg g ⁻¹ tissue)	0.6±0.03 ^a	0.6±0.2 ^a	0.5±0.06 ^a
Liver cholesterol content (mg g ⁻¹ tissue)	2.2±0.7 ^a	2.6±0.8 ^a	7.4±1.1 ^b

Notes: ¹ Values (mean±SD; n=5) in the same row with different superscripts are significantly different ($P < 0.05$).

² HSI = $100 \times \text{wet liver weight} / \text{body weight}$.

3.2 Fatty Acid Composition of Total Lipids

Fatty acids in total lipid from cod and haddock muscle decreased in the following order: polyunsaturated fatty acids (53.4%–58.7%) > saturated fatty acids (23.9%–24.9%) > monounsaturated fatty acids (15.3%–19.3%). In halibut muscle, the order was: polyunsaturated fatty acids (40.8%) > monounsaturated fatty acids (34.1%) > saturated fatty acids (23.5%). The fatty acid 22:6n-3 (DHA) was the most abundant in the total lipid of cod and haddock muscles (31.0% and 27.6%, respectively). In halibut muscle, the most abundant fatty acid was palmitic acid (16:0). The value of DHA/EPA ratio in total lipid of cod

and haddock muscle was much higher than that in halibut (2.2 and 2.3 vs 1.2, respectively) (Table 3).

Fatty acids in total lipids from fish liver decreased in the following order: monounsaturated fatty acids (41.3%–45.6%) > polyunsaturated fatty acids (29.2%–36.3%) > saturated fatty acids (20.5%–24.6%). Oleic acid (18:1n-9) was the most abundant fatty acid in total liver lipid of all the three fish species (18.1%–31.1%) (Table 3).

3.3 Fatty Acid Composition of Neutral Lipid and Polar Lipid

Fatty acid composition of neutral lipid and polar lipid extracted from fish muscle and liver is shown in Table 4.

Table 3 Fatty acid profile of fish muscle and liver total lipid^{1, 2}

Component	Muscle			Liver		
	Cod	Haddock	Halibut	Cod	Haddock	Halibut
14:0	0.6±0.1 ^a	0.5±0.1 ^a	4.9±0.3 ^b	3.5±0.8 ^c	2.4±0.2 ^d	3.8±0.2 ^e
16:0	17.7±0.3 ^a	19.2±0.5 ^b	14.6±0.3 ^c	13.6±1.2 ^d	16.7±0.8 ^e	13.5±0.6 ^d
18:0	5.0±0.3 ^a	4.6±0.1 ^b	2.8±0.1 ^c	3.7±0.5 ^d	4.4±0.8 ^b	1.8±0.1 ^e
ΣSAT ³	23.9±0.3 ^a	24.9±0.5 ^a	23.5±0.1 ^a	21.9±1.7 ^b	24.6±1.1 ^a	20.5±0.4 ^c
16:1n-7	1.4±0.1 ^a	1.2±0.2 ^a	7.9±0.5 ^d	6.8±1.2 ^b	5.5±0.4 ^c	9.2±0.5 ^e
18:1n-9	9.0±0.5 ^a	12.9±1.0 ^b	13.0±0.5 ^b	21.3±1.5 ^c	31.1±2.3 ^d	18.1±2.9 ^e
18:1n-7	2.6±0.1 ^a	3.0±0.1 ^b	2.9±0.1 ^b	4.7±0.2 ^c	4.7±0.4 ^c	3.4±0.2 ^d
20:1n-9	0.9±0.1 ^a	0.8±0.1 ^a	4.8±0.2 ^b	4.2±0.8 ^b	1.8±0.3 ^c	4.5±0.7 ^b
22:1n-11	0.2±0.0 ^a	0.1±0.0 ^a	4.1±0.3 ^b	3.0±0.4 ^c	1.1±0.3 ^d	3.4±0.7 ^e
ΣMUFA ⁴	15.3±0.7 ^a	19.3±1.2 ^b	34.1±0.6 ^c	42.0±1.6 ^d	45.6±2.1 ^e	41.3±2.8 ^d
18:2n-6	5.1±0.4 ^a	6.2±0.3 ^b	3.6±0.2 ^c	10.0±1.5 ^d	8.2±0.4 ^e	3.3±0.4 ^c
20:4n-6	2.6±0.2 ^a	2.3±0.2 ^b	1.0±0.2 ^c	0.7±0.0 ^d	0.6±0.0 ^d	0.8±0.2 ^e
Σn-6 ⁵	9.1±0.4 ^a	10.0±0.2 ^b	6.2±0.2 ^c	12.0±1.4 ^d	9.9±0.7 ^b	7.0±0.4 ^c
18:3n-3	0.4±0.1 ^a	0.3±0.0 ^b	0.9±0.1 ^c	1.3±0.2 ^d	1.0±0.0 ^e	0.5±0.1 ^e
18:4n-3	0.1±0.0 ^a	0.1±0.0 ^a	0.2±0.0 ^b	0.2±0.0 ^b	0.2±0.0 ^c	0.1±0.1 ^c
20:4n-3	0.5±0.03 ^a	0.4±0.0 ^a	0.7±0.0 ^b	0.7±0.1 ^b	0.4±0.0 ^a	1.3±0.3 ^c
20:5n-3	13.9±0.5 ^a	12.2±0.9 ^b	10.6±0.2 ^c	6.5±1.1 ^d	6.2±0.3 ^d	8.1±0.6 ^e
22:5n-3	2.2±0.1 ^a	1.6±0.1 ^b	2.2±0.1 ^a	1.5±0.3 ^b	1.2±0.2 ^c	3.1±0.2 ^d
22:6n-3	31.0±0.5 ^a	27.6±1.8 ^b	13.2±1.2 ^c	5.1±1.2 ^b	6.6±0.4 ^d	11.0±2.6 ^e
Σn-3 ⁶	48.4±0.6 ^a	42.5±1.3 ^b	30.4±0.5 ^c	16.0±2.7 ^d	16.0±0.7 ^d	26.8±2.0 ^e
ΣPUFA ⁷	58.7±0.7 ^a	53.4±1.0 ^c	40.8±0.4 ^c	32.3±1.4 ^b	29.2±1.4 ^d	36.3±2.5 ^f
DHA/EPA	2.2±0.1 ^a	2.3±0.3 ^a	1.2±0.0 ^b	0.8±0.1 ^c	1.1±0.1 ^b	1.4±0.1 ^d

Notes: ¹ Values are means of two measurements. ² Data are expressed as area percentage of FAME. ³ Including 8:0, 10:0, 12:0, 13:0, 15:0, iso16:0, 7Me16:0, 17:0, 19:0, 20:0, 21:0, 22:0, 23:0 and 24:0. ⁴ Including 14:1n-5, 14:1n-7, 15:1n-5, 16:1n-5, 17:1, 18:1n-5, 22:1n-9, 22:1n-7 and 24:1n-9. ⁵ Including 16:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 22:2n-6, and 22:5n-6. ⁶ Including 16:3n-3, 16:4n-3, 20:3n-3, 21:5n-3 and 22:4n-3. ⁷ Including 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-4, 18:2n-9, 18:3n-4, 18:4n-1, 20:2n-7 and 20:2n-9.

Table 4 Fatty acid profile of fish muscle and liver neutral lipid^{1, 2}

Component	Muscle			Liver		
	Cod	Haddock	Halibut	Cod	Haddock	Halibut
14:0	3.1±0.7 ^a	2.8±0.9 ^b	5.2±0.3 ^c	3.7±0.7 ^a	2.4±0.1 ^b	3.7±0.2 ^d
16:0	13.1±1.1 ^a	14.7±1.7 ^b	13.9±0.2 ^a	14.0±0.4 ^a	17.5±1.0 ^c	13.0±0.6 ^a
18:0	3.2±0.2 ^a	3.6±0.4 ^a	2.4±0.1 ^b	3.8±0.6 ^a	4.5±0.7 ^c	1.3±0.1 ^d
ΣSAT ³	19.5±1.2 ^a	21.5±2.0 ^b	23.1±0.4 ^c	23.0±0.3 ^c	26.1±1.6 ^d	19.1±0.8 ^a
16:1n-7	3.6±0.9 ^a	3.7±0.5 ^a	8.4±0.4 ^a	7.4±0.8 ^c	5.5±0.3 ^c	9.2±0.5 ^c
18:1n-9	16.2±3.5 ^a	20.9±1.1 ^b	14.7±0.8 ^c	22.3±1.0 ^b	31.0±1.8 ^d	18.3±3.7 ^a
18:1n-7	nd	nd	3.2±0.0 ^a	5.0±0.1 ^b	4.6±0.2 ^b	3.4±0.2 ^a
20:1n-9	2.4±0.6 ^a	1.8±0.1 ^b	5.9±0.2 ^c	4.6±1.1 ^c	1.8±0.3 ^b	4.8±1.1 ^c
22:1n-11	1.5±0.4 ^a	1.0±0.2 ^b	5.6±0.3 ^c	3.1±0.5 ^d	1.0±0.2 ^b	3.8±1.1 ^d
ΣMUFA ⁴	25.0±4.4 ^a	29.3±1.6 ^b	40.2±0.9 ^c	44.0±1.2 ^d	45.4±1.4 ^d	42.8±6.3 ^e
18:2n-6	6.2±0.8 ^a	6.8±0.5 ^a	3.8±0.3 ^b	10.4±1.4 ^c	8.1±0.5 ^c	3.6±0.5 ^b
20:4n-6	2.0±0.5 ^a	1.2±0.1 ^b	0.7±0.0 ^c	0.6±0.1 ^d	0.5±0.0 ^d	0.7±0.1 ^c
Σn-6 ⁵	9.4±1.7 ^a	9.8±1.0 ^a	6.0±0.4 ^b	12.4±1.3 ^c	9.9±0.6 ^a	6.7±1.2 ^b
18:3n-3	0.7±0.1 ^a	0.8±0.1 ^a	1.0±0.0 ^b	1.4±0.2 ^c	1.0±0.1 ^b	0.6±0.1 ^d
18:4n-3	0.6±0.1 ^a	0.5±0.3 ^a	0.2±0.0 ^b	0.2±0.0 ^b	0.1±0.0 ^b	0.2±0.1 ^b
20:4n-3	0.7±0.1 ^a	0.6±0.1 ^a	0.8±0.0 ^a	0.6±0.1 ^a	0.4±0.1 ^b	1.5±0.4 ^c
20:5n-3	14.1±2.0 ^a	10.0±0.3 ^b	9.0±0.7 ^b	6.2±1.3 ^c	5.8±0.5 ^c	8.0±0.6 ^d
22:5n-3	2.2±0.6 ^a	1.4±0.2 ^b	2.1±0.1 ^a	1.4±0.2 ^b	1.1±0.2 ^c	3.3±0.4 ^d
22:6n-3	22.8±1.8 ^a	18.0±2.0 ^b	9.4±0.6 ^c	4.5±1.6 ^e	5.9±0.4 ^e	11.3±3.0 ^c
Σn-3 ⁶	41.4±3.9 ^a	31.9±1.6 ^b	23.7±1.4 ^c	14.9±3.1 ^d	14.8±1.2 ^d	26.2±4.6 ^c
ΣPUFA ⁷	52.0±5.4 ^a	43.2±2.5 ^b	35.0±1.0 ^c	31.6±2.1 ^c	28.0±1.8 ^d	36.1±5.6 ^c
DHA/EPA	1.6±0.1 ^a	1.8±0.2 ^a	1.0±0.0 ^b	0.7±0.1 ^c	1.0±0.1 ^b	1.4±0.3 ^b

Note: Same as those of Table 3.

Fatty acids in neutral lipids from cod and haddock muscle decreased in the following order: polyunsaturated fatty acids (43.2%–52.0%)>monounsaturated fatty acids (25.0%–29.3%)>saturated fatty acids (19.5%–21.5%); DHA, oleic acid and palmitic acid were the most abundant fatty acids in cod and haddock muscle neutral lipid. In neutral lipid from halibut muscle, the order was: monounsaturated fatty acids (40.2%)>polyunsaturated fatty acids (35.0%)>saturated fatty acids (23.1%). Oleic acid was the most abundant fatty acid in halibut muscle neutral lipid, followed by palmitic acid. Fatty acids in neutral lipid from all fish liver decreased in the order: monounsaturated fatty acids (42.8%–45.4%)>polyunsaturated fatty acids (28.0%–36.1%)>saturated fatty acids (19.1%

–26.1%). Oleic acid was the most abundant fatty acid in all fish liver neutral lipid. Fatty acids in polar lipids from all fish muscle decreased in the order: polyunsaturated fatty acids (49.9%–58.1%)>saturated fatty acids (24.0%–25.7%)>monounsaturated fatty acids (14.7%–17.6%); in polar lipids from cod and haddock liver, polyunsaturated fatty acids (47%–50%)>monounsaturated fatty acids (24.7%–30.0%)>saturated fatty acids (20.8%–23.7%); In polar lipids from halibut liver, the order was: polyunsaturated fatty acids (48.9%)>saturated fatty acids (26.3%)>monounsaturated fatty acids (22.8%). All the polar lipids showed similar fatty acid patterns (Table 5). That was high in polyunsaturated fatty acids and low in Saturated fatty acids.

Table 5 Fatty acid profile of fish muscle and liver polar lipid^{1,2}

Component	Muscle			Liver		
	Cod	Haddock	Halibut	Cod	Haddock	Halibut
14:0	0.6±0.0 ^a	0.5±0.1 ^a	3.0±0.6 ^b	1.9±0.4 ^c	0.5±0.1 ^a	2.6±0.3 ^d
16:0	17.9±0.3 ^a	18.5±1.0 ^a	17.2±0.7 ^a	16.4±1.7 ^b	13.8±1.4 ^c	14.5±0.9 ^d
18:0	4.7±0.2 ^a	4.3±0.3 ^a	4.4±0.3 ^a	4.6±0.6 ^a	6.3±0.8 ^e	7.7±1.6 ^d
ΣSAT ³	24.0±0.5 ^a	24.0±1.2 ^a	25.7±0.8 ^a	23.7±2.0 ^a	20.8±1.7 ^b	26.3±1.5 ^a
16:1n-7	1.3±0.1 ^a	1.0±0.2 ^a	4.5±0.9 ^b	3.4±0.5 ^b	1.4±0.1 ^a	4.5±1.4 ^b
18:1n-9	8.7±0.5 ^a	11.9±1.5 ^b	7.9±1.0 ^a	13.2±1.3 ^b	19.9±2.0 ^c	8.6±3.0 ^a
18:1n-7	2.5±0.1 ^a	2.8±0.3 ^a	2.3±0.2 ^a	3.5±0.2 ^b	5.0±0.2 ^c	3.2±0.1 ^b
20:1n-9	0.9±0.0 ^a	0.6±0.1 ^b	2.5±0.2 ^c	1.9±0.2 ^d	1.4±0.2 ^e	2.5±0.7 ^c
22:1n-11	0.1±0.0 ^a	0.1±0.0 ^a	1.6±0.3 ^b	0.7±0.2 ^c	0.3±0.1 ^a	1.2±0.6 ^b
ΣMUFA ⁴	14.7±0.7 ^a	17.6±2.0 ^b	20.2±2.7 ^c	24.7±1.7 ^d	30.0±2.1 ^e	22.8±5.6 ^c
18:2n-6	5.0±0.4 ^a	5.8±0.5 ^a	2.4±0.2 ^b	6.8±0.8 ^c	5.6±0.4 ^a	2.6±0.2 ^b
20:4n-6	2.6±0.1 ^a	2.2±0.2 ^a	2.2±0.1 ^a	2.9±0.2 ^b	2.7±0.3 ^c	2.8±0.5 ^c
Σn-6 ⁵	9.0±0.4 ^a	9.4±0.7 ^a	6.0±0.2 ^b	11.1±0.8 ^c	9.6±0.5 ^a	7.0±0.5 ^d
18:3n-3	0.4±0.1 ^a	0.3±0.1 ^b	0.5±0.1 ^a	0.9±0.3 ^c	0.4±0.1 ^a	0.4±0.1 ^a
18:4n-3	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.1 ^a	0.2±0.0 ^b	nd	0.1±0.0 ^a
20:4n-3	0.5±0.0 ^a	0.4±0.0 ^a	0.5±0.1 ^a	0.5±0.1 ^a	0.4±0.0 ^a	0.7±0.1 ^b
20:5n-3	13.9±0.4 ^a	11.5±0.9 ^b	13.8±0.4 ^a	12.6±0.5 ^a	8.8±0.6 ^c	13.3±0.6 ^a
22:5n-3	2.1±0.1 ^a	1.5±0.1 ^b	2.2±0.1 ^a	1.9±0.3 ^a	1.2±0.1 ^c	2.6±0.2 ^d
22:6n-3	30.6±0.5 ^a	25.7±2.2 ^b	23.4±3.0 ^b	20.5±0.7 ^c	26.1±3.3 ^b	21.7±4.3 ^b
Σn-3 ⁶	48.0±0.4 ^a	39.7±2.3 ^b	41.1±2.8 ^c	37.0±0.8 ^c	37.1±3.7 ^c	39.7±4.4 ^b
ΣPUFA ⁷	58.1±0.7 ^a	49.9±2.9 ^b	50.0±2.1 ^c	50.0±0.9 ^c	47.0±3.0 ^d	48.9±4.3 ^d
DHA/EPA	2.2±0.1 ^a	2.3±0.3 ^a	1.7±0.2 ^b	1.6±0.1 ^b	3.0±0.4 ^c	1.6±0.3 ^b

Note: Same as those of Table 3.

3.4 Fatty Acid Compositions of Phospholipids

Tables 6 and 7 show fatty acid composition of cod, haddock and halibut phospholipids. Polyunsaturated fatty acids were of the highest level in phosphatidylcholine (38.7%–49.7%), followed by saturated fatty acids (32.4%–38.2%) and monounsaturated fatty acids (11.6%–23.1%). Palmitic acid was the most abundant fatty acid in phosphatidylcholine. The level of polyunsaturated fatty acids (51.9%–56.3%) in phosphatidylethanoamine was even higher than that in phosphatidylcholine. DHA was the most abundant fatty acid in phosphatidylethanoamine. Fatty acids in phosphatidylinositol decreased in the following order: polyunsaturated fatty acids (47.3%–47.5%)

>saturated fatty acids (34.4%–42.7%)>monounsaturated fatty acids (9.4%–15.4%). Stearic acid (18:0) was the most abundant fatty acid in phosphatidylinositol (20.7% in cod liver and 28.1% in halibut liver). Arachidonic acid (ArA, 20: 4n-6) was also abundant in phosphatidylinositol (13.7% in cod liver and 22% in halibut liver).

3.5 Cholesterol Content of Fish Liver and Fish Muscle

The cholesterol content of the halibut liver was much higher than those of cod and haddock liver (7.4, 2.2 and 2.6 mg g⁻¹ tissue, respectively). There was no significant difference in the cholesterol content of fish muscles among all three fish species ($P>0.05$) (Table 2).

Table 6 Fatty acid profile of fish tissue phosphatidylcholine^{1,2}

Component	Muscle			Liver		
	Cod	Haddock	Halibut	Cod	Haddock	Halibut
14:0	0.4±0.1 ^a	0.5±0.3 ^b	1.0±0.3 ^c	2.6±0.6 ^d	0.6±0.1 ^b	2.8±0.1 ^d
16:0	31.9±1.7 ^a	33.4±2.0 ^b	32.5±2.7 ^b	26.1±0.9 ^c	19.5±3.7 ^d	24.5±4.7 ^c
18:0	3.5±1.0 ^a	2.8±0.3 ^a	3.3±1.8 ^a	2.4±0.7 ^a	5.1±1.2 ^a	8.6±1.3 ^b
ΣSAT ³	37.3±0.8 ^a	37.0±2.7 ^a	38.2±3.5 ^a	32.4±1.3 ^a	29.4±0.9 ^b	37.3±3.7 ^a
16:1n-7	1.8±0.2 ^a	1.3±0.3 ^b	1.2±0.4 ^b	3.0±0.3 ^c	0.9±0.3 ^b	2.5±0.6 ^d
18:1n-9	17.7±2.4 ^a	20.7±5.1 ^b	6.5±3.4 ^c	14.0±1.2 ^b	22.4±1.6 ^b	5.1±1.5 ^c
18:1n-7	2.5±0.2 ^a	1.4±1.8 ^a	1.0±0.4 ^a	2.3±0.2 ^a	nd	2.0±0.3 ^a
20:1n-9	1.1±0.2 ^a	1.0±0.1 ^a	0.7±0.3 ^b	1.6±0.3 ^a	1.3±0.2 ^a	1.5±0.6 ^a
22:1n-11	0.3±0.1 ^a	0.2±0.1 ^b	0.2±0.2 ^b	0.7±0.3 ^b	0.5±0.1 ^b	0.4±0.2 ^b
ΣMUFA ⁴	23.0±2.6 ^a	29.0±0.8 ^a	11.6±4.1 ^b	23.1±0.5 ^a	29.5±1.4 ^a	12.7±3.1 ^b
18:2n-6	7.0±0.9 ^a	7.3±0.5 ^a	2.4±0.5 ^b	5.0±0.5 ^c	3.0±0.7 ^b	2.7±0.8 ^b
20:4n-6	1.9±0.2 ^a	1.6±0.3 ^a	2.4±0.4 ^b	1.7±0.1 ^a	0.8±0.3 ^c	1.1±0.1 ^c
Σn-6 ⁵	9.7±0.3 ^a	10.5±0.5 ^b	6.0±0.6 ^c	7.6±0.6 ^b	12.1±1.2 ^b	4.5±1.1 ^c
18:3n-3	0.4±0.1 ^a	0.3±0.1 ^a	0.1±0.0 ^b	0.5±0.1 ^a	nd	0.3±0.1 ^a
20:4n-3	0.4±0.1 ^a	0.3±0.1 ^a	0.3±0.4 ^a	0.4±0.1 ^a	0.5±0.1 ^a	0.5±0.2 ^a
20:5n-3	10.2±0.3 ^a	8.5±2.1 ^a	18.8±3.4 ^b	13.3±1.2 ^a	3.7±0.3 ^c	14±1.9 ^a
22:5n-3	1.3±0.2 ^a	1.2±0.3 ^a	1.9±0.2 ^a	1.8±0.4 ^a	5.2±0.2 ^b	2.4±0.3 ^c
22:6n-3	14.0±0.4 ^a	14.2±3.3 ^a	21.2±2.8 ^b	18.8±0.0 ^a	12.8±1.2 ^a	24.9±4.6 ^b
Σn-3 ⁶	28.3±2.9 ^a	23.7±3.1 ^b	43.4±3.9 ^c	35.2±1.3 ^d	24.6±1.3 ^b	42.3±2.8 ^c
ΣPUFA ⁷	38.7±3.0 ^a	34.0±3.5 ^b	49.7±4.2 ^c	43.8±1.8 ^c	36.7±2.1 ^b	47.8±3.0 ^c
DHA/EPA	1.5±0.1 ^a	1.6±0.3 ^a	1.2±0.3 ^a	1.4±0.1 ^a	3.5±0.2 ^b	1.8±0.6 ^a

Note: Same as those of Table 3.

Table 7 Fatty acid profile of fish tissue phosphatidylethanoamine and phosphatidylinositol^{1,2}

Component	Phosphatidylethanoamine			Phosphatidylinositol		
	Cod liver	Halibut liver	Halibut muscle	Cod liver	Halibut liver	Halibut muscle
14:0	0.8±0.6 ^a	0.6±0.1 ^a	0.4±0.0 ^a	1.1±0.9 ^a	0.7±0.0 ^a	1.9±0.8 ^a
16:0	9.5±1.9 ^a	9.7±1.3 ^a	6.0±0.6 ^a	9.6±3.4 ^a	9.1±0.8 ^a	19.6±4.4 ^a
18:0	6.4±0.4 ^a	14.2±1.3 ^b	5.8±0.8 ^a	20.7±1.3 ^a	28.1±0.5 ^b	27.3±0.6 ^b
ΣSAT ³	17.7±2.0 ^a	26.9±2.5 ^b	18.3±3.8 ^a	34.4±4.5 ^a	42.7±1.6 ^{ab}	56.3±5.3 ^b
16:1n-7	1.5±1.0 ^a	0.9±0.1 ^a	0.8±0.1 ^a	0.9±0.4 ^a	0.1±0.0 ^b	2.0
18:1n-9	10.8±1.5 ^a	5.6±0.3 ^b	4.5±1.8 ^b	7.7±0.9 ^a	4.6±0.4 ^a	6.1±1.0 ^a
18:1n-7	5.3±0.7 ^a	5.4±0.4 ^a	3.2±0.4 ^b	3.2±0.6 ^a	2.8±0.2 ^b	nd
20:1n-9	3.1±1.5 ^a	4.2±0.8 ^a	3.1±0.1 ^a	1.6±0.4 ^a	1.4±0.4 ^a	1.5±0.4 ^a
22:1n-11	1.5±1.1 ^a	0.8±0.3 ^a	0.4±0.2 ^a	0.3±0.1 ^a	0.2±0.1 ^a	3.2
ΣMUFA ⁴	23.0±3.8 ^a	19.5±1.1 ^{ab}	12.4±1.5 ^b	15.4±0.8 ^a	9.4±1.4 ^b	8.6±2.5 ^{ab}
18:2n-6	6.2±1.3 ^a	1.5±0.1 ^b	1.4±0.5 ^b	2.6±0.2 ^a	1.2±0.4 ^a	1.5±0.5 ^a
20:4n-6	2.2±0.2 ^a	1.5±0.8 ^a	2.7±0.5 ^a	13.7±2.7 ^a	22.0±2.3 ^a	6.2±2.8 ^a
Σn-6 ⁵	9.8±1.9 ^a	4.9±0.5 ^b	6.6±0.1 ^b	17.3±3.1 ^b	23.8±3.4 ^a	7.6±2.3 ^a
18:3n-3	0.5±0.1 ^a	0.1±0.1 ^a	0.8±0.7 ^a	0.3±0.1	nd	nd
20:4n-3	0.6±0.1 ^a	0.2±0.1 ^b	0.4±0.1 ^b	0.2±0.1 ^a	0.1±0.0 ^a	3.7
20:5n-3	12.8±1.3 ^a	15.6±1.6 ^a	11.1±2.5 ^a	6.1±2.0 ^a	7.2±0.2 ^a	5.7±1.9 ^a
22:5n-3	2.5±0.5 ^a	1.6±0.3 ^a	2.5±0.3 ^a	1.8±0.2 ^a	1.0±0.3 ^a	2.1±0.3 ^a
22:6n-3	28.4±2.5 ^a	27.3±1.4 ^a	39.7±1.8 ^b	18.9±3.8 ^a	13.4±1.3 ^a	12.7±1.3 ^a
Σn-3 ⁶	45.9±3.9 ^a	46.1±2.6 ^a	55.0±0.6 ^b	28.3±5.5 ^a	22.7±2.7 ^a	24.0±1.6 ^a
ΣPUFA ⁷	56.3±5.6 ^{ab}	51.9±3.4 ^a	67.5±1.9 ^b	47.3±7.2 ^a	47.5±0.5 ^a	35.1±2.9 ^a
DHA/EPA	2.2±0.1 ^a	1.8±0.2 ^a	3.7±1.0 ^a	3.2±0.4 ^a	1.9±0.2 ^a	2.3±0.6 ^a

Note: Same as those of Table 3.

4 Discussion

In the present study, although the fish were fed diets of

similar lipid sources, lipid contents of fish tissues were very different for each fish species. Haddock liver had significantly high lipid content, which was almost double that of cod and halibut liver. The result for haddock liver

lipid level (67.2%) in the present study was in agreement with the previously published data (63.2%–69.0%, Nanton *et al.*, 2001). However, lipid content of cod liver (36.9%) in our study was lower than that (50%–60%) reported by Lie *et al.* (1986), and lipid content of halibut liver (30.7%) in this study was higher than that (7%–19%) reported by Hatlen *et al.* (2005).

Fish can be divided into three broad categories based on the lipid content of their flesh: lean, moderate-fat and high-fat fish. Cod and haddock are typical lean fish; lipid content of their muscle was lower than 1% in the present study, which agreed well with the previous data (Santos *et al.*, 1993; Nanton *et al.*, 2001). Halibut is a low-fat fish and is ranked between lean fish and medium-fat fish like Atlantic salmon (Ackman, 1989). The value of halibut muscle lipid content in the present study was in agreement with the recorded data for farmed halibut (3.5%–7.4%), but much higher than those of wild halibut (0.1%–0.15%) (Olsson *et al.*, 2003).

Lean fish flesh does not represent an energy reserve organ like fat fish (Addison, 1968). In fat fish flesh, triglycerides are the major lipid form, but the structural phospholipids exceeding the high energy triglycerides in lean fish muscle has been observed in previous work (Ackman, 1989; Santos *et al.*, 1993; Nanton *et al.*, 2001). Results of this study were in accordance with those previously reported. As lean fish, 79% of total lipid from cod muscle and 92.9% of total lipid from haddock muscle were phospholipids. Unlike cod and haddock, both halibut muscle and liver were dominated by neutral lipid in the present study, which was also in agreement with Olsson *et al.* (2003) who reported that farmed halibut muscle fat was dominated by triglycerides, but wild halibut muscle fat was mostly made up of structural phospholipids.

In the present study, the largest amount of fatty acid group in neutral lipid from cod and haddock muscle was polyunsaturated fatty acids. However, monounsaturated fatty acids were the most abundant in neutral lipid from halibut muscle, which agreed well with the findings of Addison (1968), Lie *et al.* (1986) and Polvi and Ackman (1992). Their investigations indicated that the major form of neutral lipid in lean fish muscle was sterol esters which showed a much higher polyunsaturated fatty acid level. Triglyceride, the major lipid form in neutral lipid extracted from fat fish muscle, was especially low in polyunsaturated fatty acids and high in monounsaturated fatty acids.

Cholesterol analysis in the present study also confirmed the difference in lipid class composition of cod/haddock muscle and halibut muscle; 53.3% of neutral lipid in muscle of cod and 66.6% of neutral lipid in muscle of haddock were cholesterol, while only 0.86% of neutral lipid from halibut muscle was cholesterol.

Phosphatidylcholine and phosphatidylethanolamine normally represent the largest phospholipid fractions in fish tissues and often reflect the pattern of dietary fatty acids (Bell *et al.*, 1986). In the present study, phosphatidylcholine was the major phospholipid group in all fish tissues and was the only fraction that could be separated

from cod and haddock muscle sample. Halibut muscle was the only one we could separate all the phospholipid groups, including phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidic acid. In cod and halibut liver, phosphatidylethanolamine and phosphatidylinositol were the other two phospholipid group which could be separated besides phosphatidylcholine. Compared with phospholipid from halibut tissue, oleic acid and 18:2n-6 levels in cod and haddock phosphatidylcholine and phosphatidylethanolamine were higher, reflecting the difference in fatty acid composition of dietary lipid. However, even if fish tissue phospholipids reflected dietary fatty acid profile to a certain extent, each phospholipid group had its own characteristic. Polyunsaturated fatty acids were abundant in phosphatidylcholine and phosphatidylethanolamine. Phosphatidylethanolamine appeared to be particularly rich in EPA and DHA content. The percentage of ArA was the highest in phosphatidylinositol. These results agreed well with those previously reported on dogfish, *Scyliorhinus canicula*, cod, *Gadus morhua* (Bell *et al.*, 1983; Bell and Tocher, 1990) and rainbow trout, *Salmo gairdneri* (Tocher, 1993). The relatively constant phospholipid composition in fish tissue indicates that phospholipids play a key role in the maintenance of metabolic and structural function in fish (Sargent *et al.*, 1989; Tocher *et al.*, 2008).

5 Conclusion

In conclusion, cod and haddock were shown to be lean fish, whose liver stored most lipid of the body, and structural phospholipid was the major lipid form of their flesh. However, as a medium-fat fish, halibut used both liver and muscle as the lipid storage organs.

Acknowledgements

The authors wish to thank Mr. Sean M. Tibbetts for his excellent technical assistance and Dr. Wenshan Liu for his assistance in English version.

References

- Ackman, R. G., 1989. Nutritional composition of fats in seafoods. *Prog. Food Nutr. Sci.*, **13**(3-4): 161-241.
- Ackman, R. G., 1998. Remarks on official methods employing boron trifluoride in the preparation of methyl esters of the fatty acids of fish oils. *J. Am. Oil Chem. Soc.*, **75**: 541-545.
- Addison, R. F., 1968. Distribution of fatty acids in cod flesh lipids. *J. Fish Res. Board Can.*, **25**: 2083-2090.
- AOAC, 1990. *Official Methods of Analysis*. 15th edition. Association of Official Analytical Chemists, Washington, D. C., 1094-1098, 1298.
- Bell, M. V., and Tocher, D. R., 1990. Molecular species composition of phosphatidylinositol from brain, retina, liver and muscle of cod (*Gadus morhua*). *Lipids*, **25**: 691-694.
- Bell, M. V., Henderson, R. J., and Sargent, J. R., 1986. The role of polyunsaturated fatty acids in fish. *Comp. Biochem. Physiol.*, **83B**(4): 711-719.
- Bell, M. V., Simpson, C. M. F., and Sargent, J. R., 1983. (n-3)

- and (n-6) polyunsaturated fatty acids in the phosphoglycerides of salt-secreting epithelia from two marine fish species. *Lipids*, **18**: 720-725.
- Bligh, E. C., and Dyer, W. J., 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**: 911-927.
- Ebling, M. E., 1968. The Dumas method for nitrogen in feeds. *J. Assoc. Off. Anal. Chem.*, **51**: 766-770.
- Folch, J., Lees, M., and Sloane-Stanley, G. H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biochem.*, **226**: 497-509.
- Hatlen, B., Grisdale-Helland, B., and Helland, S. J., 2005. Growth, feed utilization and body composition in two size groups of Atlantic halibut (*Hippoglossus hippoglossus*) fed diets differing in protein and carbohydrate content. *Aquaculture*, **249**: 401-408.
- Juaneda, P., and Rocquelin, G., 1985. Rapid and convenient separation of phospholipids and non-phosphorus lipids from rat heart using silica cartridges. *Lipids*, **20**: 40-44.
- Kovacs, M. I. P., 1979. A simple method for the determination of cholesterol and some plant sterols in fishery-based food products. *J. Food Sci.*, **44**: 1299-1231.
- Lie, Ø., Einar, L., and Georg, L., 1986. Liver retention of fat and of fatty acids in cod (*Gadus morhua* L.) fed different oils. *Aquaculture*, **59**: 187-196.
- Nanton, D. A., Lall, S. P., and McNiven, M. A., 2001. Effects of dietary lipid level on liver and muscle lipid deposition in juvenile haddock, *Melanogrammus aeglefinus* L. *Aquac. Res.*, **32** (Suppl. 1): 225-234.
- Olsen, R. E., and Henderson, R. J., 1989. The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. *J. Exp. Mar. Biol. Ecol.*, **129**: 189-197.
- Olsson, G. B., Olsen, R. L., Carlehog, M., and Ofstad, R., 2003. Seasonal variations in chemical and sensory characteristics of farmed and wild Atlantic halibut *Hippoglossus hippoglossus*. *Aquaculture*, **217**: 191-205.
- Polvi, S. M., and Ackman, R. G., 1992. Atlantic salmon (*Salmo salar*) muscle lipids and their response to alternative dietary fatty acid sources. *J. Agric. Food Chem.*, **40**: 1001-1007.
- Santos, J. D., Burkow, I. C., and Jobling, M., 1993. Patterns of growth and lipid deposition in cod (*Gadus morhua* L.) fed natural prey and fish-based feeds. *Aquaculture*, **110**: 173-189.
- Sargent, J. R., Henderson, R. J., and Tocher, D. R., 1989. The lipids. In: *Fish Nutrition*. Halver, J. E., ed., Academic Press, New York, 154-218.
- Tocher, D. R., 1993. Incorporation and metabolism of (n-3) and (n-6) polyunsaturated fatty acids in phospholipid classes in cultured rainbow trout (*Salmo gairdneri*) cells. *Fish Physiol. Biochem.*, **8**: 239-249.
- Tocher, D. R., Bendiksen, E. A., Campbell, P. J., and Bell, J. G., 2008. The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture*, **280**: 21-34.
- Vitiello, F., and Zanetta, J. P., 1978. Thin-layer chromatography of phospholipids. *J. Chromatogr.*, **166**: 637-640.

(Edited by Qiu Yantao)