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Variation in total lipid content and fatty acid composition in the muscle of Bombayduck *Harpodon nehereus* (Hamilton, 1922) with respect to size and season

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ABSTRACT

In view of the increased importance of fish lipids owing to their human health beneficial effects, the total lipid content and fatty acid composition in the muscle of Bombayduck (*Harpodon nehereus*) of different sizes harvested in different seasons were studied. Among the three seasons studied, total muscle lipids were significantly ($p<0.05$) high during winter and low in monsoon season. In all the seasons, the increased lipid levels were noticed with increase in size. A significant variation in total lipid was observed with the lowest level (1.65 %) in small sized Bombayduck of monsoon season and the highest level (4.68 %) was observed in large sized fish caught in winter season. A total of 37 fatty acids encompassing n-3, n-5, n-6, n-7, n-9 and odd and branched chain fatty acids were characterised with relatively high levels (26.57 – 35.03%) of n-3 polyunsaturated fatty acids (PUFA) and a favourable ratio (3 to 4.69) of n-3/n-6 PUFA demonstrating excellent nutritional quality of Bombayduck. Though the fatty acid composition was not influenced by the size of fish, the effect of season on fatty acid composition of Bombayduck was clearly established by principal component analysis.

Keywords: Bombayduck, Fatty acid profile, Lipid contents, Seasonal variation

Introduction

Fish lipids assume importance because of their high levels of n-3 (omega-3) polyunsaturated fatty acids (PUFA), which have been reported to reduce the risk of coronary heart diseases (Erkkila *et al.,* 2003) and lower blood pressure and plasma triacylglycerol levels (Dallongeville *et al.,* 2003). In addition, they can reduce the symptoms of diabetes (James *et al.,* 2000) as well as a range of other disorders (Nichols *et al.,* 2002). Lipid content and fatty acid composition in fish are known to vary significantly depending on the season, location, availability of food items and environmental conditions such as temperature, salinity and pressure (Exler *et al.,* 1975; Bandara *et al.,* 1997). Previous studies have shown that n-3 PUFA of marine animals vary depending on various biological and environmental factors, such as taxonomy, diet of the animals, water temperature and the latitude at which they were harvested (Dunstan *et al.,* 1999).

Bombayduck (*Harpodon nehereus)* is one of the important marine pelagic species accounting 6% of the total pelagic finfish resources of India. It is an important fishery especially along the west coast of the country with total production of 1,12,279 t (CMFRI, 2011). The nutritional value of Bombayduck has been investigated earlier by Gopakumar (1997). Although the fatty acid profiles and lipid content of fresh and dried Bombayduck have been reported (Joydeep *et al.,* 1999), there is no information available on seasonal changes in the total lipid content and fatty acid composition with respect to different size groups. As Bombayduck is an important species with high consumption rate in fresh condtion along the Mumbai coast, the present work was undertaken to have a better understanding of the nutritive lipid and fatty acid composition in the muscle tissue and also to investigate the changes in the total lipid content and fatty acid composition of the species at different sizes caught during different seasons.

Materials and methods

Samples were collected from Versova Landing Centre, along Mumbai coast during monsoon (August), winter (December) and summer (April) seasons. The morphometric data of fishes selected for different size groups is presented in Table 1. Fishes were classified as small, medium and large groups based on their average length and weight.

Table 1. Morphometric data of different size groups of Bombay duck (*Harpodon nehereus)* sampled for the study

Size	Body length (cm)	Body weight (g)
Small	10-15	0-50
Medium	15-20	50-200
Large	>20	> 200

Lipid extraction

Total lipid was extracted from muscle and liver following Folch (1957). Muscle tissue (5 g) was homogenised in 10 volume of methanol (w/v) followed by 20 volume of chloroform (w/v) in a homogeniser (ART Miccra, Germany). The homogenate was filtered (using a funnel with a folded defatted filter paper) to recover the liquid phase and the filter residue was re-homogenised with a second volume of chloroform–methanol. The filtrate was washed with 0.2 volume (4 ml for 20 ml) of 0.9% NaCl solution and phases were vigorously mixed. The mixture was poured into a separating funnel and allowed to separate. The lower chloroform phase containing lipids was collected and evaporated under vacuum in a rotary evaporator to bring down the volume to 2-3 ml. Further evaporation of chloroform was done under nitrogen stream and residue was weighed to quantify the amount of lipid extracted. The lipid residue was re-dissolved in chloroform/ methanol (2:1, v/v) and then stored in a 25 ml conical flask with glass stopper under nitrogen at -20 °C until needed.

Preparation of fatty acid methyl esters (FAME)

The method as per AOAC (1995) was followed to esterify the lipid. FAME was prepared from the isolated lipids by heating with the methanolic NaOH and then with BF_3 methanol for esterification. An aliquot of 5 ml n-heptane was added to recover the methyl esters in organic phase. The mixture was washed with saturated NaCl solution and two phases were separated using a separating funnel. The upper n-heptane phase was collected and stored in 10 ml all glass vials until further analysis.

Gas chromatography – mass spectrometry (GC-MS)

Fatty acid methyl esters were separated using a Shimadzu QP2010 quadruple Gas Chromatography Mass Spectrometer (GCMS) equipped with a Carbowax (30 m x 0.25 mm ID; 0.25-µm film thickness) capillary column (Cromlab S.A.). Helium was used as the carrier gas. Injector and detector temperatures were set at 250 °C. Injection was performed in split mode (1:15). The column temperature was programmed initially at 50 °C for 2 min and then increased at a rate of 10 °C per min to a final temperature of 230 °C. FAME was separated at constant pressure (23.1 kpa) and peaks were identified by comparing standard mass spectra with the relative abundances of *m/z* ranging from 40 to 550. The values of fatty acids are presented in peak area percentage of total identified fatty acids.

Statistical analysis

Analysis of variance (ANOVA) was performed on total lipid content data and significance differences at $p<0.05$ betweem mean values were evaluated using Duncan's test with multiple comparison by employing one way ANOVA with SPSS statistical package. The multivariate data of fatty acid composition subjected to different size and seasons were analysed by principal component analysis (PCA) using software Unscrambler (Version 9.5, CAMO, Norway). PCA is a data compression method based on the correlation among variables. The purpose of carrying out PCA was to express the main information in the variables by a lower number of variables, the so-called principal components (PC1, PC2,…). Score plots from the PCA explored the main trends in data, and their respective loadings revealed the significant fatty acids. Both the scores and loadings were explained by the bi-plot.

Results and discussion

Total lipid content

The data on total lipid content (%) recorded in the muscle tissue of different size groups of Bombayduck during different seasons are shown in Table 2. Significant differences ($p<0.05$) were observed during different seasons with respect to different sizes. The total lipid content in muscle was found to be in the range of 1.65 – 4.68%. There are a number of classifications available by which fishes are divided into groups according to their lipid content. According to the one proposed by Kleimenov (1971), Bombayduck belongs to the group of low fat fishes having an average lipid content of 2-8%. The highest concentration (4.68±0.89%) of lipids was recorded in winter compared to other seasons. In muscle, higher percentage of lipid content was found in large size fish which was caught during winter, whereas, the lowest (1.65±0.10%) amount was observed in small size fish during monsoon compared to the other size groups. During the winter season, the lipid contents in the body of fishes as well as prey animals have been reported to be very high (Tanaka, 1980). As the fish consume prey with high lipid content, the additional lipid would be deposited in the body of fishes resulting in higher lipid levels during winter.

Table 2. Total lipid content (%) in the muscle tissue of different size groups of Bombayduck, recorded during different seasons

Seasons	Size group		
	Small	Medium	Large
Monsoon	1.65 ± 0.10^{aA}	1.90 ± 0.12^{aB}	2.71 ± 0.05^{aC}
Winter	2.03 ± 0.31^{bA}	3.25 ± 0.89^{cB}	4.68 ± 0.99^{cC}
Summer	1.76 ± 0.21^{aA}	2.89 ± 0.59^{bB}	4.17 ± 0.89^{bC}

Valuses are Mean± SD

Superscripts a, b and c represents significant differences ($p< 0.05$) among same sizes in different seasons (Mean± SD). A, B and C represent significant differences ($p< 0.05$) among different sizes in each season.

Fatty acid composition

A total of 37 fatty acids were identified in muscle tissue of three different sizes of Bombayduck during the three different seasons and the data are presented in Table 3. From the fatty acid data, total saturated fatty acids (SAFA), total monounsaturated fatty acids (MUFA), sum of n-6 fatty acids (Σ n-6), sum of n-3 fatty acids (Σ n-3) and n-6/ n-3 ratio were calculated. In the earlier studies on fatty acids

Table 3. Fatty acid composition (% of total fatty acids by peak area) of muscle tissue in different size groups (a: small, b: medium, c: large) of Bombayduck caught during the three seasons

Fatty acids	Monsoon			Winter			Summer		
	a	b	c	a	b	c	a	b	c
6:0	0.03	0.04	0.09	0.01	0.02	0.01	0.04	0.07	0.06
8:0	0.05	0.08	0.05	0.02	0.03	0.02	0.06	0.90	0.07
12:0	0.33	0.44	0.23	0.61	0.42	0.39	0.22	0.15	0.26
13:0	0.03	0.06	0.02	0.07	0.05	0.05	0.05	0.09	0.05
14:0	5.66	4.64	4.97	4.79	5.88	6.18	3.01	2.64	3.21
i-15:0	0.07	0.16	0.19	0.18	0.15	0.15	0.12	0.11	0.14
15:0	0.82	1.07	0.52	1.18	0.81	0.92	1.04	0.73	1.21
i-16:0	0.08	0.13	0.79	0.43	0.80	0.14	0.11	0.17	0.09
16:0	19.10	14.59	18.14	19.48	17.55	17.86	14.45	18.77	17.06
i-17:0	0.11	0.46	0.17	0.75	0.45	0.48	0.22	0.12	0.31
17:0	1.24	1.51	0.77	1.67	1.72	1.47	1.52	1.15	1.34
i-18:0	0.09	0.30	0.45	0.31	0.78	0.24	0.14	0.15	0.18
18:0	8.70	8.62	8.86	9.43	9.00	8.82	11.16	10.44	12.21
19:0	0.32	0.57	0.11	0.35	0.42	0.37	0.33	0.26	0.42
20:0	0.63	0.85	0.34	0.72	0.49	0.67	0.25	0.43	0.26
23:0	0.28	0.38	0.14	0.62	0.33	0.18	0.21	0.20	0.34
Total SAFA	37.54	33.9	35.84	40.62	38.90	37.95	32.93	36.38	37.21
16:1n-9	0.25	0.25	1.57	1.65	1.38	1.10	1.26	1.08	1.04
16:1n-7	9.37	7.51	8.36	7.53	9.50	9.52	4.31	5.21	3.26
18:1n-9	12.24	10.23	13.82	9.93	11.30	11.95	13.43	10.81	12.35
18:1n-7	3.76	3.20	2.45	3.30	3.41	3.46	6.01	3.73	4.50
18:1n-5	0.07	0.11	0.06	0.09	0.94	0.20	0.42	0.13	0.27
20:1n-9	1.28	0.88	0.62	0.41	0.94	1.22	0.74	0.68	0.56
20:1n-7	0.27	0.32	0.09	0.87	0.29	0.42	0.16	0.12	0.22
22:1n-9	0.22	0.21	0.37	0.20	0.14	0.15	0.10	0.05	0.14
Total MUFA	27.46	22.71	27.34	23.98	27.90	28.02	26.43	21.81	22.34
18:2n-6	0.95	1.12	1.20	1.40	0.86	0.95	1.31	1.14	1.20
20:2n-6	0.26	0.38	0.35	0.21	0.41	0.32	0.32	0.28	0.36
20:3n-6	0.12	0.32	0.14	0.72	0.44	0.19	0.06	0.08	0.10
20:4n-6	4.34	4.73	4.26	5.08	3.76	4.03	4.52	5.88	5.31
22:4n-6	0.37	0.44	0.21	0.38	0.26	0.14	0.22	0.33	0.40
22:5n-6	1.29	1.37	1.06	1.04	0.84	1.10	0.71	0.97	0.52
Total n-6 PUFA	7.33	8.36	7.22	8.83	6.57	6.73	7.14	8.68	7.89
18:3n-3	0.28	0.51	0.18	0.67	0.32	0.24	0.24	0.43	0.28
18:4n-3	0.23	0.43	0.32	0.27	0.24	0.25	0.12	0.14	0.10
20:3n-3	0.07	0.12	0.11	0.69	0.52	0.11	0.05	0.03	0.04
20:4n-3	0.14	0.38	0.15	0.31	0.16	0.28	0.53	0.23	0.45
20:5n-3	6.26	10.07	8.74	6.82	5.77	6.04	6.14	8.07	6.78
22:5n-3	2.34	2.57	1.49	1.44	2.11	1.89	1.21	1.91	1.05
22:6n-3	18.35	20.95	18.61	16.37	17.51	18.49	25.21	22.32	23.86
Total n-3 PUFA	27.67	35.03	29.6	26.57	26.63	27.30	33.50	33.13	32.56
n3/n6 ratio	3.77	4.19	4.09	3.00	4.05	4.05	4.69	3.81	4.12

profiles of Bombayduck, 15 fatty acids were identified by Gopakumar (1997) and 19 were reported by Joydeep *et al.* (1999). In the present study, use of capillary column for resolution of components and MS (Mass spectrometer) for identification of fatty acids enabled us to characterise 37 fatty acids encompassing n-3, n-5, n-6, n-7, n-9 and odd and branched chain fatty acids (OBCFA). Total polyunsaturated fatty acids (n-6 and n-3) were found higher than monounsaturated fatty acids in the present study.

In muscle tissue, the most common SAFA were palmitic acid (14.45-19.10%) and stearic acid (8.62-12.21%). Among MUFA, oleic acid (18:1n-9) was the most abundant comprising of 9.93-13.82% of the total fatty acids followed by palmitoleic acid (16:1n-7) which formed 3.26-9.37%. Arachidionic acid (AA, 20:4n-6) was the principal n-6 PUFA at a level of 3.76-5.88% of total fatty acids. Eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were the major n-3 PUFA identified, accounting 5.77-10.07, 1.05-2.57 and 16.37-25.21% respectively. The main OBCFA identified in the present study were isomers of pentadecanoic acid (*iso* C15:0), hexadecanoic acid (*iso* C16:0), heptadecanoic acid (*iso* C17:0) and octadecanoic acid (*iso* C18:0).

Marine fishes have been reported to possess substantial amount of human health beneficial fatty acids *viz*., arachidonic acid, EPA and DHA. It has also been reported that lipid content and fatty acid composition changes according to species, diet, geographical origin and season (Tashiro *et al.,* 1981; Hatano, 1990). In addition to the seasonal variations, body size, location and sexual maturity also have been reported to influence the fatty acid composition (Ackman, 1982). In general, the fatty acid composition of fish is mainly influenced by their prey animals (Watanabe, 1982). On the other hand, fishes generally change prey according to their own body size (Tanaka, 1980).

The edible portion of the Bombayduck muscle tissue recorded high levels of PUFA which may be attributed to their feeding, as they depend entirely on marine food chain, which are known sources of long chain PUFA. It has been reported that the muscle of larger pelagic fish has greater concentration of EPA and DHA compared to smaller fish (Pozo *et al.,* 1992). In contrast to earlier reports, no significant variation was observed among different sizes of Bombayduck in the present study. Pigott (1989) suggested that the n-3/n-6 ratio is a better index in comparing relative nutritional value of fish. In the light of the diversified roles of n-3 and n-6 PUFA, right balance between these fatty acid groups is recommended. It has been reported that high levels of n-6 PUFA in the human diet can lead to many health disorders (Sargent and Tacon, 1999). Whereas, n-3 PUFA have been reported to modulate the undesirable effects of n-6 PUFA. In this background, the observed high ratio of n-3/n-6 PUFA in the present study (3 to 4.69) emphasises the excellent quality of Bombayduck meat.

The fatty acid composition of fish in general is known to be affected by diet (Olsen and Skjervold, 1995), food deprivation (De Silva *et al.,* 1997) and non-dietary factors including environmental temperature (Bell *et al.,* 1986). It has been reported that the PUFA content in fish and shellfish varies inversely with water temperature, while the SAFA content changes positively with water temperature (Dunstan *et al.,* 1999). Environmental temperature is known to affect the fatty acid composition of the tissue of poikilothermic fish in that the degree of unsaturation increases with decreasing temperature (Morris and Culkin, 1989). In contrast to the earlier studies, PUFA content increased during summer in Bombayduck, may be due to its cannibalistic feeding habits and due to the unavailability of marine algae in the food chain during summer (Pillay and Hora, 1953; Govindan, 1972). Though the total lipid content in the muscle tissue recorded was low, the concentration of EPA and DHA which are considered as human health beneficial fatty acids (Joydeep *et al.,* 1999), observed were very high. Small size Bombayduck had lowest lipid contents compared to the other size groups during all the seasons. However, all the size groups of Bombayduck harvested during the different seasons possessed relatively high proportion of nutritionally important PUFA with highly favourable n-3/n-6 ratio.

Identification of iso-fatty acids

Besides linear fatty acids, branched-chain saturated fatty acids were identified by mass spectrometry of the methyl ester derivatives based on the molecular ions (m/z value), especially *iso* fatty acids such as *iso*-15:0, *iso*-16:0, *iso*-17:0 and *iso*-18:0, which were found in the range of 0.07 to 0.80%. Vlaeminck *et al.* (2006) suggested that the polyunsaturated and odd and branched chain fatty acids (OBFCA) of marine food chain received considerable attention because of their various biological activities in health and diseases. These fatty acids may occur in small amounts and have been detected in the range of 0.1-1% each (Ackman, 1976; Young, 1982). The OBCFA in fish products are of further interest because of their low melting points relative to chain length (Enser, 1984). Other reasons for interest in odd and branched chain fatty acids are their reported anticarcinogenic effects. Marine fishes do not have capacity to synthesise odd and branched chain fatty acids and they derive these entirely from the marine food chain (Ackman *et al*., 1980). In the present study, OBCFAs did not show any specific trend either with respect to size or season.

Multivariate statistical analysis has been applied for fish species in respect of fatty acids data, which reduces the dimensionality of multivariate data while preserving most of the variance within it (Eriksson *et al.,* 2001). Such methods have been successfully applied in lipid research in fishes like tilapia and rays (De Silva *et al.,* 1997; Ould El Kebir *et al.,* 2003). In view of the large data comprising 9 samples and 37 variables, principal component analysis (PCA) was employed in the present study to observe the relationships among different seasons and sizes with respect to fatty acids. A graphic representation of the projection of variables and samples onto the first two principal components is given in Fig. 1 in the form of bi-plot.

The distribution of different fatty acids with respect to different seasons and sizes was evident in the bi-plot (Fig. 1). The Bombayduck samples were segregated into clusters mainly based on season rather than size demonstrating the profound effect of season on fatty acid profile compared to the size of fish. PC1, which explained 31% of total information in the data, has clearly separated summer from other seasons. Further, it can be clearly noticed from the bi-plot that, the Bombayduck caught in summer season were rich in DHA, stearic acid and 18:1n-7.

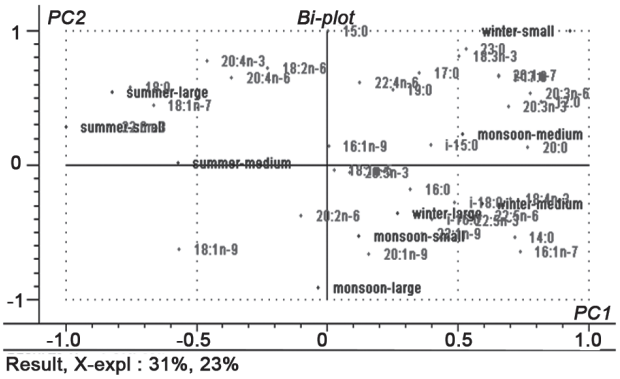

Fig. 1. Muscle fatty acid distribution in Bombayduck with respect to size and season

It can be concluded from the present study that the lipid content of Bombayduck muscle significantly ($p<0.05$) varied among different seasons. The fish harvested in winter season has more lipid content in muscle compared to the fish caught in other seasons irrespective of the body size. Bombayduck, an important pelagic marine fishery resource of India, recorded high levels of n-3 PUFA, which are good sources of human health promoting fatty acids such as EPA and DHA. The PCA carried out in the present study demonstrated that it is an important tool to investigate the relationship among fish samples of different sizes caught in different seasons with respect to their fatty acid compositions.

References

Ackman, R. G. 1976. Fish oil composition. In: *Objective methods for food evaluation*. National Academy of Sciences, Washington D.C., p. 103-131.

Ackman, R.G., Sebedio, J. L. and Kovacs, M. I. P. 1980. Role of eicosenoic and docosenoic fatty acids in freshwater and marine lipids. *Mar. Chem.,* 9: 157-164.

Ackman, R. G. 1982. Origin of marine fatty acids in nutritional evaluation of long chain fatty acids. In: Barlow S. M. and Stansby M. E. (Eds.). *Fish oil*. Academic Press, London, p. 25-88.

AOAC, 1995. *Official methods of analysis of Association of Official Analytical Chemists*, Volume I, 16th edn. AOAC International, Arlington, USA.

Bandara, N. M., Batista, I., Nunes, M. L., Empis, J. M. and Christie, W. 1997. Seasonal changes in lipid composition of sardine (*Sardina pilchardus*). *J. Food Sci*., 62:40 –42.

Bell, M. V., Henderson, R. J. and Sargent, J. R. 1986. The role of polyunsaturated fatty acids in fish. *Com. Biochem. Physiol.*, 83B: 711–719.

CMFRI, 2011. *CMFRI Annual report 2010-11*, CMFRI, Cochin

Dallongeville, J., Yarnell, J., Ducimetiere, P., Arveiler, D., Ferriers, J., Montaye, M., Luc, G., Evans, A., Bingham, A. and Hass, B. 2003. Fish consumption is associated with lower heart rates. *Circulation*, 108(7): 820–825.

De Silva, S. S., Gunasekera, R. M., Collins, R., Ingram, B. A. and Austin, C. M. 1997. Changes in the fatty acid profile of the Australian short fin eel in relation to development. *J. Fish Biol.*, 50 (5): 992–998.

Dunstan, G. A., Olley, J., Ratkowsky, D. A. 1999. Major environmental and biological factors influencing the fatty acid composition of seafood from Indo-Pacific to Antarctic waters. *Rec. Res. Dev. Lipid Res.,* 3: 63–86.

Enser, M. 1984. The chemistry, biochemistry and nutritional importance of animal fats. In: Wiseman, J., Butterworths (Ed.), *Fats in animal nutrition,* London, UK, p. 23–54.

Erkkila, A. T., Lehto, S., Pyorala, K. and Uusitupa, M. I. 2003. N-3 Fatty acids and 5-y risks of death and cardiovascular disease events in patients with coronary artery disease. *Amer. J. Clinical Nutr.,* 78: 65–71.

Eriksson, L., Johansson, E., Kettaneh-Wold, N. and Wold, S. 2001. *Multi - and megavariate data analysis.* Umetrics Academy, Umea, Sweden.

Exler, J., Kensella, J. E. and Watt, B. K. 1975. Lipids and fatty acids of important finfish: new data for nutrient tables. *J. Amer. Oil Chem. Soc*., 52: 154-159.

Folch, J. L., Sloane, M. and Stanley, G. H. S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.,* 226: 497–509.

Gopakumar, K. 1997. *Indian food fishes: Biochemical composition*. Central Institute of Fisheries Technology. Indian Council of Agricultural Research, Cochin, India, p. 22- 28.

Govindan, T. K. 1972. *Harpodon nehereus*. *Sea Food Export J.*, 6: 21-24.

Hatano, M. 1990. Lipids. In: Takeuchi K. K. M. (Ed.), *The nutritive components of fish and new fish utilization technology*, Tokyo, p. 34-43.

James, M. J., Gibson, R. A. and Cleland, L. G. 2000. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Amer. J. Clinical Nutr.*, 71: 343S–348S.

Joydeep, D., Debasish, P., Bhattacharya, A., Chakraborty, J., Banerjee, D. and Ghosh, A. 1999. Comparative study on fatty acids and either lipids of fresh and sun-dried Bombayduck (*Harpadon nehereus*). *J. Food Sci. Technol.,* 36 (6): 506-510.

Kleimenov, I. Y. 1971. *Importance of fish as food*. Moscow, Nauka.

Morris, R. J. and Culkin, F. 1989. Fish. In: Ackman R. G (Ed.), *Marine biogenic lipids, fats and oils,* CRC Press, Boca Raton, Florida, p. 145-178.

Nichols, P. D., Mooney, B. D. and Elliott, N. G. 2002. Nutritional value of Australian seafood II. Factors affecting oil composition of edible species, *Report ofr the Fisheries Research and Development Corporation*. CSIRO Marine Research, Hobart, Australia. 199 pp.

Olsen,Y. and Skjervold, H. 1995. Variation in content of ω3 fatty acids in farmed Atlantic salmon, with special emphasis on effects on non-dietary factors. *Aquacult. Int.,* 3: 22–35.

Ould El Kebir, M. V., Barnathan, G., Siau, Y., Miralles, J. and Gaydou, E. M. 2003. Fatty acid distribution in muscle, liver and gonads of rays (*Dasyatis marmorata, Rhinobatos cemiculus* and *Rhinoptera marginata*) from the East Tropical Atlantic Ocean. *J. Agri. Food Chem.,* 51: 1942–1947.

Pillay T. V. R. and Hora, S. L. 1953. The food and feeding habits of the Bombayduck, *Harpodon nehereus* (Ham.) in the river Matlah (Bengal). *Proc. Nat. Inst. Sci. India*, 19, 427-435.

Pigott, G. M. 1989. The need to improve omega-3 content of cultured fish. *World Aquacult. Magaz.*, 20: 63–68.

Pozo, R., Perez-Villarreal, B. and Saitua, E. 1992. Total lipids and omega-3 fatty acids from seven species of pelagic fish. In: Burt, J. R., Hardy, R. and Whittle, K. J. (Eds.), *Pelagic fish - The resources and its exploitation*. Fishing News Books, London, p. 142-147.

Sargent, J. R. and Tacon, A. 1999. Development of farmed fish: a nutritionally necessary alternative to meat. *Proc. Nutr. Soc.,* 58: 377- 383.

Tanaka, S. 1980. Biological research on salmon sharks (*Lamna ditropis*). *Report on the research of new resources development on shark*, Japan Marine Fishery Resources Research Center, p. 59-84.

Tashiro, I., Itoh, S. and Tsuyuki, H. 1981. Seasonal variation of lipids of horse mackerel. *Nip. Su. K. Ga.,* 28: 309–317.

Vlaeminck, B., Fievez, V., Cabrita, A. R. J., Fonseca, A. J. M. and Dewhurst, R. J. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. *Animal Feed Sci. Technol.,* 131(3-4): 389-417.

Watanabe, T. 1982. Lipid nutrition in fish. *Comp. Biochem. Physiol.,* 73B: 3–15.

Young, F. V. K. 1982. The production and use of fish oils. In: Barlow, S. M. and Standsby, M. E. (Eds.), *Nutritional evaluation of longchain fatty acids in fish oils*. Academic press, London, p. 1-23.

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