



# Profiling of Omega-3 Polyunsaturated Fatty Acids of Myctophid Fish Species Available in Arabian Sea

R. Navaneethan, S. Vimaladevi, K. K. Ajeesh Kumar, R. Anandan\*, S. C. Niladri, K. K. Asha and Suseela Mathew

ICAR-Central Institute of Fisheries Technology, P.O Matsyapuri, Cochin - 682 029, India

## Abstract

Nutritional database on omega-3 polyunsaturated fatty acids profile of myctophid fish species from Arabian Sea is relatively scanty. In this study, the omega-3 fatty acids [docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)] profile of four myctophid species of fishes (*Benthoosema pterotum*, *Benthoosema fibulatum*, *Diaphus jenseni*, *Myctophum spinosum*) caught from Arabian Sea was compared with commerson's anchovy (*Stolephorus commersonii*) to evaluate their nutritional quality for human health benefits. The protein content of myctophid fishes ranged between 19.36% for *Myctophum spinosum* to 13.74% for *Benthoosema pterotum*. Total fat (3.64%) and moisture content (81.03%) were high in *Benthoosema pterotum* (3.64%). *Stolephorus commersonii* was found to have higher ash content (3.23%). Myctophid omega-3 PUFA profile is comparable to Commerson's anchovy fatty acid composition. Presence of higher DHA content in *M. spinosum* (20.2%), *B. pterotum* (17.48%) was comparable to edible *S. commersonii* (16.9%) emphasizes that these underutilized non-edible myctophids may be considered as a potential nutrient resource of omega-3 PUFA especially DHA for the formulation of nutraceuticals, cosmetics and animal feeds.

**Keywords:** Myctophids, omega-3 PUFA, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), human healthcare

## Introduction

Seafood is one of the main sources of long chain omega 3 fatty acids that include Eicosapentaenoic Acid (EPA), and Docosahexaenoic Acid (DHA).

(EPA; C20:5) and (DHA; C22:6). Consumption of EPA and DHA reduce the risk of cardiovascular disease and sudden cardiac death and are associated with several other health benefits (Skeaff & Miller, 2009; Kris-Etherton et al., 2003; Yokoyama et al., 2007). Clinical investigations in infants indicate that DHA is essential for normal functional development of retina and brain, particularly in premature newborns (Conner, 2000). The predominant sources of very long-chain  $\omega$ -3 PUFA are fish and fish oil supplements (Sahena et al., 2010). Myctophids (lanternfish) are widely distributed in the world's oceans and contribute to a large proportion of the deep-sea fish biomass (Hasegawa et al., 2008). The myctophid family comprises of 230–250 species arrayed within 30–35 genera (Paxton, 1972). Myctophid fishes are reported to contain high amounts of wax esters and when consumed in large quantities, cause diarrhoea and seborrhoea in animals (Lekshmy et al., 1983). Several studies reported that myctophid fishes are good source of protein and minerals and are similar to other common marine fishes except for the fact that they have very high fat content ranging from 4.9 to 28.5% (Gopakumar et al., 1983; Noguchi, 2004). Rajamoorthy et al. (2013) studied proximate composition of *Diaphus watasei*, and reported the moisture, fat, protein and ash content as 63.19, 15.13, 21.40 and 1.33% respectively. These studies clearly indicate the nutritional potential of this species for human consumption and formulation of novel foods. Commerson's anchovy (*Stolephorus commersonii*), is one of the common pelagic fishes used for human consumption. In this present study, an attempt was made to investigate the proximate composition and omega 3 fatty acid profiles of selected myctophid fishes and compare the same with Commerson's anchovy.

## Materials and Methods

Around 250 g of selected Myctophid species of fishes namely *M. spinosum*, *D. jenseni* *B. fibulatum*,

Received 23 May 2015; Revised 20 August 2015; Accepted 10 October 2015

\* E-mail: kranandan@rediffmail.com

and *B. pterotum* of length (mm) and weight (g) around 89 and 6.5, 69 and 3.0, 59 and 2.5 and 40 and 1.5 respectively, were collected from the FORV Sagar Sampada cruise operated off Lakshadweep and Cochin coast during October 2013. Anchovy (*S. commersonii*) samples of weight and length 85 and 4.6 respectively were bought from a local fish market in Cochin. Fish samples were obtained under iced condition in styrofoam boxes and transported to the laboratory in insulated container with ice.

Moisture content was determined according to the AOAC (2000) method. About 15 g of homogenized sample was dried in a hot air oven at 110°C until constant weight was obtained. Ash content was determined by placing the sample for 12 h in a furnace at 525°C (AOAC, 2000). Total protein content in the homogenized samples (0.2 g) was determined using Kjeldahl method (AOAC, 2000). Results were expressed as percentage of wet weight basis. Total lipids were extracted using chloroform/methanol (2:1) (Folch et al., 1957). Aliquots of the chloroform layer extract were evaporated to dryness under nitrogen and the lipids were quantified gravimetrically.

About 100 mg of lipid sample from the fish source was saponified by refluxing for about 5 min with 5 ml of 0.5 N methanolic NaOH and the esterification was done by refluxing with Boron trifluoride-methanol. Upon cooling saturated NaCl was added. Finally, the fatty acids solution were extracted with petroleum ether and washed with water. The solvent was evaporated and made up to 1 ml.

Fatty acid methyl esters were analysed by using Perkin Elmer Clarus 580 Gas Chromatograph (Norwalk, CTO 6859, USA). Peaks were identified by comparison of their retention times with total (fatty acid methyl esters) FAMES standards. Results were expressed as percentage.

Analyse were performed in triplicate and averaged. Results were expressed as mean  $\pm$  SD and Duncan's test was used to assess statistical significance.

## Results and Discussion

Seafood is one of the main sources of omega 3 fatty acids (Ortiza et al., 2011). According to the environment conditions, feeding habits, age, maturity and species, the fatty acid composition may vary in fish (Celik et al., 2005; Halilogulu et al., 2004). Fatty acids and their biological effects were well documented and reviewed by Horrocks & Yeo (1999). The proximate composition and fatty acid components (saturated fatty acid (SFA), mono unsaturated fatty acid (MUFA) and poly unsaturated fatty acid (PUFA) were extracted from four myctophid species and compared with that of anchovy. About 19.36% of protein, was found in *Myctophum spinosum* followed by *Diaphus jenseni* (18.85%), *Benthosema fibulatum* (18.6%), *Stolephorus commersonii* (17.48%) and *Benthosema pterotum* (13.74%). Total fat was high in *Benthosema pterotum* (3.64%) which also was high in moisture content (81.03%). *Stolephorus commersonii* was found to have higher ash content (3.23%).

Saturated fatty acid was found to be the predominant among all the fishes except *B. fibulatum* (Table. 2). Comparatively good amount of stearic acid was found in in *D. jenseni* (18.10%), *M. spinosum* (15.60%) and *S. commersonii* (10.28%). *B. pterotum* contains around 16.5% of arachidic acid.

Among MUFA *S. commersonii* and *D. jenseni* contains high amount of oleic acid at 18.19 and 9.47% respectively. Oleic acid is known to promote the production of antioxidants and to slow down the development of heart disease (Waterman & Lockwood, 2007). In *M. spinosum* pentadecenoic acid (5.42%) and palmitoleic acid (5.21%) were the predominant monounsaturated fatty acids, whereas

Table 1. Comparison of proximate composition in myctophid and anchovy (*S. commersonii*)

Species	Myctophid fishes				Anchovy
	<i>B. pterotum</i>	<i>B. fibulatum</i>	<i>D. jenseni</i>	<i>M. spinosum</i>	<i>S. commersonii</i>
Moisture	81.0 $\pm$ 0.38 <sup>d</sup>	76.6 $\pm$ 0.05 <sup>b</sup>	76.9 $\pm$ 0.15 <sup>b</sup>	77.6 $\pm$ 0.3 <sup>a</sup>	77.6 $\pm$ 0.34 <sup>c</sup>
Protein	13.7 $\pm$ 0.17 <sup>a</sup>	18.6 $\pm$ 0.11 <sup>c</sup>	18.8 $\pm$ 0.21 <sup>c</sup>	19.3 $\pm$ 0.21 <sup>d</sup>	17.4 $\pm$ 0.21 <sup>b</sup>
Fat	3.64 $\pm$ 0.13 <sup>d</sup>	3.26 $\pm$ 0.07 <sup>c</sup>	3.43 $\pm$ 0.19 <sup>c,d</sup>	2.36 $\pm$ 0.08 <sup>b</sup>	1.86 $\pm$ 0.08 <sup>a</sup>
Ash	3 $\pm$ 0.11 <sup>b</sup>	3.09 $\pm$ 0.37 <sup>b</sup>	3 $\pm$ 0.18 <sup>b</sup>	2.32 $\pm$ 0.15 <sup>a</sup>	3.23 $\pm$ 0.11 <sup>b</sup>

Table 2. Comparison of fatty acid composition in four myctophid fishes and anchovy (*S. commersonii*)

Species	Myctophid fishes				Anchovy
	<i>B. pterotum</i>	<i>B. fibulatum</i>	<i>D. jenseni</i>	<i>M. spinosum</i>	<i>S. commersonii</i>
SFA	58.76±0.15	44.85±0.05	65.25±0.04	56.42±0.11	42.81±0.18
MUFA	8.46±0.04	4.56±0.02	16.74±0.05	11.14±0.02	27.51±0.05
PUFA	32.35±0.03	50.47±0.04	16.97±0.04	30.95±0.04	28.29±0.07

Table 3. Comparison of PUFA (%) in four myctophid fishes and anchovy

Species	Myctophid fishes				Anchovy
	<i>B. pterotum</i>	<i>B. fibulatum</i>	<i>D. jenseni</i>	<i>M. spinosum</i>	<i>S. commersonii</i>
PUFA					
C 18:2	0.42±0.02 <sup>a</sup>	16.8±0.02 <sup>e</sup>	1.76±0.02 <sup>c</sup>	1.94±0.03 <sup>d</sup>	1.67±0.03 <sup>b</sup>
C 18:3	2.21±0.01 <sup>d</sup>	9.14±0.05 <sup>e</sup>	1.67±0.01 <sup>c</sup>	0.24±0.02 <sup>a</sup>	1.31±0.03 <sup>b</sup>
C 20:2	1.76±0.02 <sup>e</sup>	1.27±0.03 <sup>d</sup>	0.36±0.02 <sup>b</sup>	-	0.57±0.08 <sup>c</sup>
C 20:3	2.16±0.07 <sup>d</sup>	0.82±0.03 <sup>b</sup>	1.26±0.02 <sup>c</sup>	2.48±0.07 <sup>e</sup>	0.16±0.01 <sup>a</sup>
C 20:4	1.36±0.01 <sup>d</sup>	0.86±0.01 <sup>c</sup>	0.23±0.02 <sup>b</sup>	-	2.77±0.15 <sup>e</sup>
C 20:5	6.96±0.02 <sup>d</sup>	6.09±0.03 <sup>c</sup>	4.08±0.06 <sup>a</sup>	6.09±0.06 <sup>c</sup>	4.91±0.07 <sup>b</sup>
C 22:6	17.4±0.05 <sup>c</sup>	15.4±0.11 <sup>b</sup>	7.61±0.12 <sup>a</sup>	20.2±0.02 <sup>d</sup>	16.9±0.09 <sup>b,c</sup>

in *B. pterotum* eicosenoic acid was found to be about 6.36%.

PUFA content was found to be high in *B. fibulatum* (50.61%) compared to other fishes. EPA and DHA, which play important roles in treatment of cardiovascular diseases and in infant brain and retina development, were found to be rich in *B. fibulatum* (Conner, 2000).

DHA is found to be predominant polyunsaturated fatty acid in *M. spinosum* (20.2%), *B. pterotum* (17.48%) and *S. commersonii* (16.9%). A comparable amount of DHA was also present in *B. fibulatum* (15.52%) and a reasonable amount of EPA and DHA in *D. jenseni* (Fig. 1). The other major fatty acids were linoleic acid (16.87%) and linolenic acid (9.19%) found in *B. fibulatum*.

In spite of their presence in abundant amount in the seas around India, myctophid fishes are the least studied and underutilized. It is important to explore its potential for utilizing myctophid species especially in view of their nutritional value. Fatty acid profile presented here has revealed the nutritional significance of *B. fibulatum*, *D. jenseni*, *B. pterotum* and *M. spinosum* and compares well with fatty acid

composition of *S. commersonii*. Presence of significantly higher level of DHA, docosahexaenoic acid (C22:6) a fatty acid with potential health significance in the myctophid fishes studied may be considered as a viable source for the extraction and purification of DHA, which is essentially required for the development of fetus brain and retina during pregnancy. Myctophids contains high content of triglycerides that serve primarily as an energy store and also known to have wax esters, mainly used for buoyancy (FAO, 1997). Fats act as sources of

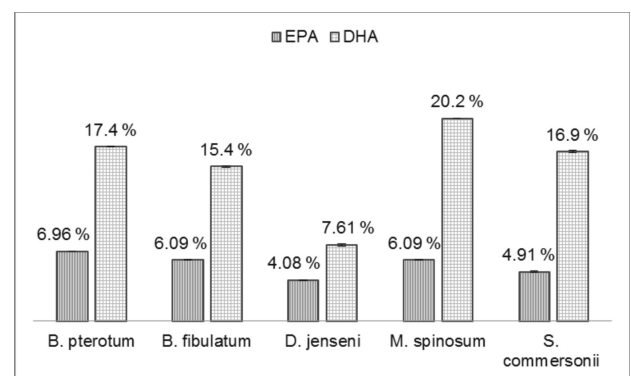


Fig. 1. EPA and DHA content in myctophid fishes and anchovy

essential fatty acids required for the structural and functional integrity of organisms (Piggott & Tucker, 1990). Since myctophids are not utilized for direct human consumption so far, it may be used as a potential nutrient resource in the formulation of pharmaceutical products and animal feed. Further studies will be carried out for the characterization of wax esters in myctophid fishes.

### Acknowledgements

The study was carried out as part of the project "Myctophid Resource assessment in the Arabian Sea and Development of Harvest and Post-harvest Technologies" financed by Centre for Marine Living Resources & Ecology, Ministry of Earth Science, Govt. of India and the financial assistance is gratefully acknowledged. The authors are thankful to the Director, Central Institute of Fisheries Technology (ICAR), for providing the necessary facilities and granting permission to publish this paper.

### References

- AOAC (2000) Official Methods of Analysis. 17<sup>th</sup> edn., Association of official Analytical Chemists International, North Fredrick Avenue, Gaithersburg, Maryland, USA
- Çelik, M., Diler, A. and Küçükgülmez, A. (2005) A comparison of the proximate compositions and fatty acid profiles of zander (*Sander lucioperca*) from two different regions and climatic conditions. *Food Chem.* 92: 637-641
- Conner, W.E. (2000) Importance of n-3 fatty acids in health and disease. *Am. J. Clin. Nutr.* 71: 171S-175S
- FAO (1997) Review of the state of world fishery resources: marine fisheries. FAO Fisheries Circular No. 920, FAO, Marine Resource Service, Fisheries Resources Division, Fisheries Department. FAO Fisheries Circular No. 920, Rome, FAO, 173 p
- Folch, J., Lees, M., Stanely, G.H.S. (1957) A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497-509
- Gopakumar, K., Nair, K. G. R., Nair, P. G. V., Nair, A. L., Radhakrishnan, A. G. and Nair, P. R. (1983) Studies on lantern fish *Benthosemapterotum* biochemical and microbiological investigations. *Fish. Technol.* 20: 17-19
- Haliloglu, H. I., Bayir, A., Sirkecioglu, A. N., Aras, N. M. and Atamanalip, M. O. (2004) Comparison of fatty acid composition in some tissues of rainbow trout (*Oncorhynchus mykiss*) living in seawater and freshwater. *Food Chem.* 86: 55-59
- Hasegawa, E. I., Sawada, K., Abe, K., Watanabe, K., Uchikawa, K., Okazaki, Y., Toyama, M. and Douglas, R. H. (2008) The visual pigments of a deep-sea myctophid fish *Myctophum nitidulum* Garman; an HPLC and spectroscopic description of a non-paired rhodopsin-porphyrin system. *J. Fish Biol.* 72: 937-945
- Horrocks, L.A. and Yeo, Y.K. (1999) Health benefits of docosahexaenoic acid. *Pharmaceut. Res.* 40: 211-225
- Kris-Etherton, P.M., Harris, W. S. and Appel, L.J. (2003) Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. *Arterioscler. Thromb. Vasc. Biol.* 23: 20-30
- Lekshmy, N. A., Arul, J. M., Mathew, P. T. and Gopakumar, K. (1983) Studies on lantern fish (*Benthosemapterotum*) II. Nutritional Evaluation. *Fish. Technol.* 20: 17-19
- Noguchi, S.F. (2004) Utilization of the resources of lantern fish as fisheries Products, In: *More Efficient Utilization of Fish and Fishery Products. Developments in Food Science 42* (Sakaguchi, M., Ed), p. 63-74, Elsevier, Amsterdam
- Paxton, J. R. (1972) Osteology and relationships of the lanternfishes (Family Myctophidae). *Bulletin of the Natural History Museum of Los Angeles County* 13: 1- 81
- Piggott, G. M. and Tucker, B. W. (1990) The effect of technology on nutrition. Marcel Dekker Inc. 362
- Rajamoorthy, K., Pradeep, K., Anandan, R., Libin Baby, Sankar, T. V. and Lakshmanan, P. T. (2013) Biochemical Composition of Myctophid Species *Diaphuswatasei* and *Myctophum obtusirostre* Caught from Arabian Sea. *Fish. Technol.* 50: 41-44
- Sahena, F., Zaidul, I. S.M., Jinap, S., Yazid, A.M., Khatib, A. and Norulaini, N. A. N. (2010) Fatty acid compositions of fish oil extracted from different parts of Indian mackerel (*Rastrelliger kanagurta*) using various techniques of supercritical CO<sub>2</sub> extraction. *Food Chem.* 120: 879-885
- Skeaff, C.M. and Miller, J. (2009) Dietary fat and coronary heart disease: summary of evidence from prospective cohort and randomised controlled trials. *Ann. Nutr. Metab.* 55: 173-201
- Waterman, E. and Lockwood, B. (2007) Active components and clinical applications of olive oil. *Alternat. Med. Rev.* 12(4): 331-342
- Ortiz, X. Carabellido, L. M. Martí, R. Martí, X. Tomás, and Díaz-Ferrero, J. (2011) Elimination of persistent organic pollutants from fish oil with solid adsorbents. *Chemosphere* 82: 1301-1307
- Yokoyama, M., Origasa, H., Matsuzaki, M., Matsuzawa, Y., Saito Y. (2007) Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* .369: 1090-1098