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Note

Proximate composition and fatty acid profile of the myctophid *Diaphus watasei* Jordan & Starks, 1904 from the Arabian Sea

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ABSTRACT

The edible portion of *Diaphus watasei,* a benthopelagic fish collected off Quilon, south-west coast of India was analysed for proximate composition and fatty acid profile. The fleshy part of the fish was found to contain 15.62% protein, 11.71% fat, 0.47% minerals, 0.28% soluble carbohydrate and 0.01% crude fibre. The dry matter in the fish was found to be about 28%. Monounsaturated fatty acids (MUFA) were found to have the highest (36.7%) share among total fatty acids followed by saturated fatty acids (SFA) (33.3%) and polyunsaturated fatty acids (PUFA) (25.5%). The abundant fatty acids were found to be oleic acid, palmitic acid, docosahexaenoic acid, stearic acid, myristic acid, linolenic acid, eicosapentaenoic acid and palmitoleic acid. The most predominant fatty acid was recorded as oleic acid which contributed 32% to the total fatty acids. Docosahexaenoic acid formed the single largest component of PUFA (9.33%) followed by γ linolenic acid (3.97%) and eicosapentaenoic acid (3.83%). The ω-3 PUFA contributed about 70% of the total PUFAs. The most important ω-3 PUFAs were EPA and DHA that contributed 73% to the total ù -3 polyunsaturated fatty acids.

Keywords: Arabian Sea, *Diaphus watasei*, Fatty acid profile, Myctophids, Proximate composition

Fishes constitute a major portion of the deepsea shrimp trawl by-catch, which demands only very low price and are often discarded in the sea at the time of catch/sorting. These by-catches, when landed are mostly used for fishmeal or manure production. Along the south-west coast of India, lantern fish (myctophiformes) forms a major portion (20-35%) of the fish by-catch in the deepsea shrimp trawls (Bineesh *et al*., 2009). GLOBEC (1993) revealed the existence of large quantity of myctophid stock in the Arabian Sea which is mainly contributed by a single species, *Benthosema pterotum,* which has an estimated biomass of 100 million t in the world oceans. GjØsaeter and Kawaguchi (1980) reported that Indian Ocean has a rich fauna of lanternfishes both in number of species and biomass. Wide occurrence of *Diaphus* spp. from the eastern and north-eastern Arabian Sea has been reported (FAO, 1997; Balu and Menon, 2006). A study carried out by the Central Marine Fisheries Research Institute (CMFRI), during 1997-2002, estimated a biomass of 100,000 t of myctophids along the Indian EEZ of Arabian Sea, dominated by *Diaphus* sp. (Balu and Menon, 2006). Blindheim *et al*. (1975) reported a good concentration of myctophids from certain parts of the south-west coast of India and stated that they had been commercially exploited at certain localities. There are only a few examples of commercial exploitation of lanternfishes; fishermen in Suruga Bay, central Japan used large quantities of *Diaphus* spp. as food (Kubota, 1982). Commercial fishery for *D. coeruleus* and *Gymnoscopelus nicholski* (edible species) in the south-west Indian Ocean and southern Atlantic began in 1977 and catch by former USSR countries reached 51,680 t in 1992, after which the fishery ceased. Despite this, the Commission for Conservation of Antarctic Marine Living Resources (CCAMLR) still permits a 2,00,000 t TAC (Total Allowable Catch) for this fishery in its jurisdiction area. An industrial purseseine fishery for *Lampanyctodes hectoris* in South African waters closed in the mid 1980s due to processing difficulties caused by the high oil content of the fish (FAO, 1997). Polyunasaturated fatty acids (PUFAs) are important structural components of cell membranes, and are useful in growth and development of human beings (Chakraborty *et al*., 2010). PUFAs, especially of longer chain length, *viz*., EPA and DHA were reported to be found abundant in marine fish, and have beneficial properties to the prevention of atherosclerosis and other diseases. Therefore, newer species of marine fishes need to be explored as potential healthy food items for better nutrition and health.

Attempts were made in India to utilise fish from the shrimp trawler by-catch, effectively by formulating various products acceptable to consumers (Gopakumar *et al*., 1976), however no detailed biochemical studies with respect to proximate and fatty acid composition have been carried out on deepsea myctophids to understand their nutritional value and use as healthy food items. An understanding of the proximate composition and fatty acids of the species is important, particularly during processing and product development. The study presents the proximate composition and fatty acid profile of one of the dominant deepsea myctophid species, *D. watasei*.

Specimens of *D. watasei* were collected from commercial deepsea shrimp trawlers operated off Quilon, along the south-east coast of Arabian Sea (9-11º N; 72-76º E) during September 2009 at depths of 250 - 400 m. *D. watasei* is a small fish, usually attain a maximum size of 15 - 30 cm with elongate and slightly compressed body. They are similar to anchovies in appearance. Fish samples were transported to laboratory in insulated container with ice and maintained at -22 ºC. Fishes ranging in size from 70 - 130 mm SL (standard length) and 10 - 24 g wet weight were used for analysis. The samples were cleaned in distilled water and scales as well as skin were removed before analysis. Skeletal muscles from dorsal fin to caudal fin of the body were collected and minced well in a mixer to get a homogenous sample.

Proximate composition analysis

Moisture content of the samples was analysed by drying the samples of known weight at 60 ^{0}C in hot air oven overnight until constant weight, followed by drying for 2 h in a hot air oven at 100 ^{0}C (AOAC, 2006). Total nitrogen content (crude protein) was determined by micro Kjeldhal method and the result was multiplied by 6.25 to arrive at the crude protein percentage. Fat was estimated by Soxhlet apparatus using petroleum ether (60 - 80 ^{0}C boiling point). Crude fiber is determined following the fraction remaining after refluxing with standard solution of H_2SO_4 (1.25% w/v) and NaOH (1.25% w/v) for 30 min, under controlled condition. Acid insoluble ash and nitrogen free extract were determined following AOAC (2006) methods.

Fatty acid analysis

The fatty acid composition of the sample was determined as described by Bligh and Dyer (1959) with suitable modification (Chakraborty *et al*., 2007). Fat extracted using methanol : chloroform mixture (2:1, v/v), were saponified and trans-esterified yielding fatty acid methyl esters (FAME). These esters were extracted with n-hexane/water mixture (1:2, v/v). After removal of the aqueous layer, the n-hexane layer was passed through Na_2SO_4, concentrated *in vacuo*, reconstituted in petroleum ether, and stored at -20 °C until required for analyses. A Perkin Elmer Auto System XL, Gas chromatograph (Perkin Elmer, USA) equipped with a flame ionisation detector (FID) was used for analysis of the fatty acids. The esterified fatty acids were analysed by gas liquid chromatography with FID detector and compared with fatty acid methyl ester standards (Supelco FAME 37 standard).

Proximate composition

The average meat yield from *D. watasei* was 47.25%. The proximate composition of the fish muscle is given in Table 1. The mean moisture content of the meat was 72% of the wet body weight. The protein (15.62% wet weight) and fat (11.71% ww) contents were fairly high. The mean ash content was 0.47% of w/w.

Table 1. Proximate composition of *D. watasei* (on % wet weight basis)

Parameters *	Wet tissue weight (%)
Dry matter	28.09 $\pm$ 0.04
Crude protein	15.62 $\pm$ 0.08
Crude fat	11.71 $\pm$ 0.09
Crude ash	0.47 $\pm$ 0.03
Crude fiber	0.01 $\pm$ 0.00
Acid insoluble ash	Negligible
Soluble carbohydrate	0.28 $\pm$ 0.02

* AOAC (2006)

Fatty acid composition

The fatty acid composition of the fish muscle showed more unsaturated fatty acids (62.2%) than saturated fatty acids (33.28%) (Fig. 1). Major fatty acids present in the fish muscle are given in Table 2. The monounsaturated fatty acids (MUFA) formed the major fatty acids (36.66%) followed by saturated fatty acids (SFA) 33.28% and polyunsaturated fatty acids (PUFA) 25.54%. The percentage of monoenes, dienes and polyenes was 36.66%, 1.49% and 24.05%, respectively of the total fatty acids. The more abundant fatty acids were oleic acid, palmitic acid,

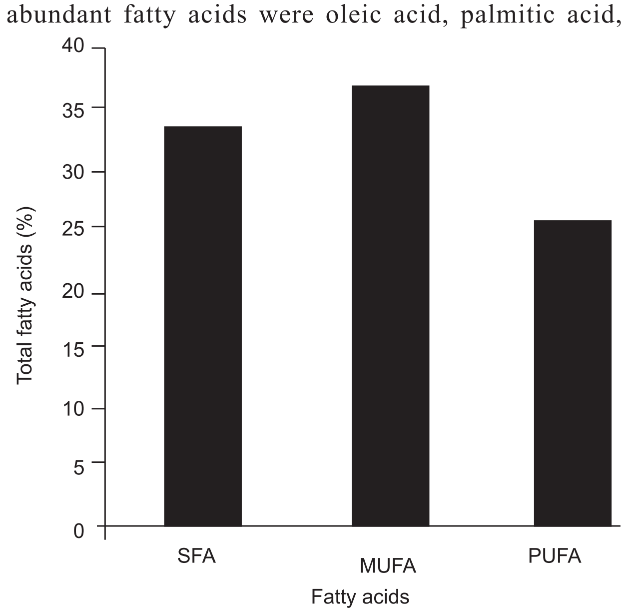

Fig. 1. Percentage of muscle fatty acids of *D. wataesi*

docosahexaenoic acid, stearic acid, myristic acid, linolenic acid, eicosapentaenoic acid and palmitoleic acid. Oleic acid (C18:1n9, 32.03%) was found to be the most prominent. Among the saturated fatty acids palmitic acid (C16:0, 18.03%) was found to be the principal fatty acid. Docosahexaenoic acid (DHA) (C22:6n3, 9.33%), linolenic acid, both γ (GLA) and α (ALA) (C18:3n6 and C18:3n3, 3.97% and 3.47%, respectively) and eicosapentaenoic acid (EPA) (C20:5n3, 3.83%) constitute the principal components of PUFA (Fig. 2.). The fish contain 18.09% of ω-3 fatty acids and 7.45% of ω-6 fatty acids. ω-3 PUFA contributed nearly 70% of the total PUFAs. The most important ω-3 PUFAs, namely EPA and DHA contributed to 73% of the total ω-3 polyunsaturated fatty acids. The fish had a higher ω-3/ω-6 ratio of 2.43.

Table 2. Fatty acid composition of *D. watasei*

Fatty acids	Total fatty acids (%)
Saturated fatty acids (SFA)	
C12:0	0.05 ± 0.00
C14:0	4.42 ± 0.03
C15:0	0.47 ± 0.02
C16:0	18.30 ± 0.92
C17:0	0.77 ± 0.05
C18:0	9.22 ± 0.06
C22:0	0.05 ± 0.00
Total SFA	33.28
Monounsaturated fatty acids (MUFA)	
C14:1n7	0.10 ± 0.01
C16:1n7	3.68 ± 0.02
C18:1n9	32.03 ± 1.27
C24:1	0.85 ± 0.0
Total MUFA	336.66
Polyunsaturated fatty acids (PUFA)	
C18:2n6	1.05 ± 0.21
C18:3n6	3.97 ± 0.13
C18:3n3	3.47 ± 0.16
C20:2n6	0.44 ± 0.04
C20:3n6	0.33 ± 0.02
C20:4n6	1.66 ± 0.09
C20:5n3	3.83 ± 0.21
C22:5n3	1.46 ± 0.08
C22:6n3	9.33 ± 0.17
Total PUFA	25.54

Increase in human population and an increased demand for low value food fishes to meet the nutritional requirement has made myctophids a possible potential resource for exploitation and utilisation. Studies have revealed the abundance of *Diaphus* spp. in the eastern and north-eastern Arabian Sea, where the concentration of *Benthosema pterotum* increases along the Pakistan coast (FAO, 1997) Observations from the present study also indicate that *D. watasei* dominated the trawl catch along the south-west coast of India. Several studies have assayed the chemical composition of myctophids and other mesopelagic fishes (Ayyappan *et al*., 1976; Menon, 1976; Gopakumar *et al*., 1983; Ackman, 1990; Suriah *et al*., 1995; Seo *et al*., 1996; Phleger *et al*., 1999; Seo *et al*., 2001). From nutritional point of view, myctophids are high in proteins, variable in lipids and uniformly low in carbohydrates. Studies have evaluated the lipid content of vertically migrating myctophids and found that they include both triglycerides, which is believed to serve primarily as an energy store, and wax esters, mainly used for buoyancy (FAO, 1997). Gopakumar *et al*. (1983) attempted the prime work in myctophids from the Arabian Sea and estimated biochemical composition of, *B. pterotum* from the Gulf of Oman and Aden, and reported 16.1 g protein per 100 g wet tissue weight and 3.4 g fat per 100 g wet tissue weight. They concluded that lantern fish resembles most marine fish with regard to biochemical composition and can be used as food for both human and animals. Seo *et al*. (1996) analysed lipid level in myctophids as 0.5 - 21.7% of total weight. Suda (1973) reported myctophids had oil extraction efficiency of 110 lt^{-1}. Myctophids are essentially crustacean zooplankton feeders and the environmental conditions *viz*., temperature, salinity, availability of food in different seasons *etc* have significant influence on the proximate composition of fish. Ayyappan *et al*. (1976) estimated protein, lipid and ash content of miscellaneous edible fish

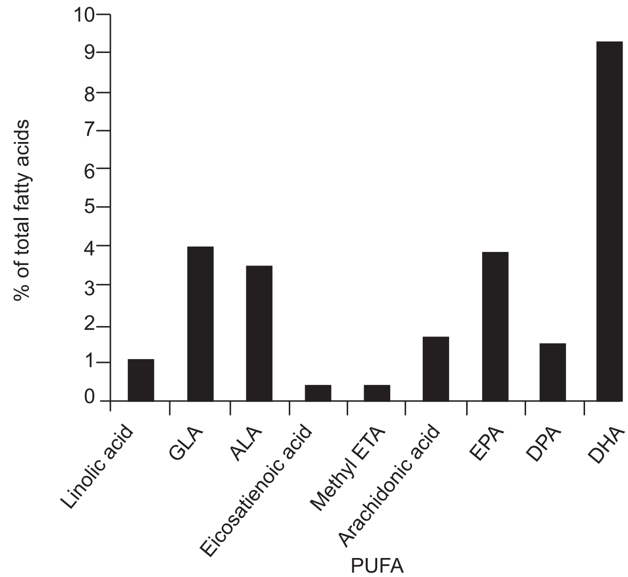

Fig. 2. Percentage of PUFA with respect to total muscle fatty acids in *D. watasei*

from shrimp trawlers and recorded a range of protein from 16.02 - 20.77%, lipid from 0.3 - 5.31% and ash from 3.2 - 5.6%; most of the species recorded high lipid content and low moisture content. In the present study, the protein composition recorded was within the range reported for edible fish (15.62% ww), whereas lipid content registered higher value (11.71% ww) and mineral content was very meagre (0.47% ww).

Fishes are often classified on the basis of their fat content into lean (fat content below 5%), medium fat (5 -10%) and fatty fish (>10%) (Suriah *et al*., 1995). According to Ackman (1990), fishes can be classified as high fat fish where average fat content is more than 8%. Fairly high levels of fat makes myctophids a 'fatty species' and the taste of the fish seems to be largely depending on the fat content. There may be slight difference in the lipid levels and fatty acid content in the same species depending on the sex, age, size, maturity, season, food availability, geographical variation, salinity and water temperature (Stansby, 1981; Piggott and Tucker, 1990). Phleger *et al*. (1999) stated that most of the body lipids of myctophids are stored in the flesh of the fish (68 - 92%). Earlier works (Seo *et al*., 1996; Saito and Murata, 1996; Phleger *et al*., 1999) revealed that myctophids have high content of monoene fatty acids in the lipids and the present study also supports this. Oleic acid is the major fatty acid followed by palmitic acid which is similar with the study by Saito and Murata (1996). Study by Seo *et al*. (1996) proved that palmitic acid and DHA were the major fatty acids in tropical myctophids where as oleic acid and palmitic acid were predominant in the temperate water species, though oleic acid was the major fatty acid of total lipids in myctophids. Fatty acid profile of *Diaphus theta* and *Diaphus gigas* (Saito and Murata, 1996) from the northern Pacific Ocean showed high levels of monounsaturated fatty acids followed by saturated fatty acids and polyunsaturated fatty acids, but both the species showed low level of PUFA (*D. theta*, 20.7% and *D. gigas,* 9.6% of total fatty acids) as compared with that of the present study with an average PUFA of 25.54%. This is not in agreement with the report by Ackman (1989) that the tropical and sub-tropical species are reported to contain lower levels of PUFA than temperate species. Phleger *et al*. (1999) studied the composition of wax ester and triacylglycerol in eleven species of myctophids and found that, wax ester dominated species contained lower levels of PUFA than in triacylglycerol-rich species and estimated a high amount of triacylglycerol with an average of 74% of total lipids in *Diaphus* spp. The total ω-3 fatty acids were found to be higher than the ω-6 where, this is factual in most of the marine fishes especially DHA and EPA (Wang *et al*., 1990). As in the case of marine fishes, this species is also rich in DHA, EPA and ALA, which are the major components of omega 3 fatty acid which is one of the essential nutrient for humans.

Diaphus watasei has high protein as well as fat content and hence it could well be a potential source of alternative protein and fat. At present *D. watasei* is not commercially exploited in India, although it is used for preparing fish meal by selected local populations. Since *D. watasei* has high fat content, there is a possibility of fatty acid rancidity during the production of fish meal and hence before processing oil can be extracted so that rancidity can be minimised. Also the fish is not suitable for long time freezing and cold storage due to fatty acid oxidation. In view of the beneficial nutritive value, *D. watasei* is suitable for direct consumption. Since most myctophid resources are subjected to non-predatory mortality, with the biomass ending up with decomposition, attempts can be made to encourage targeted exploitation of these valuable resource which can be utilised as a source of cheap animal protein.

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