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Inter-annual variability and seasonal dynamics of amino acid, vitamin and mineral signatures of ribbon fish, *Trichiurus lepturus* (Linnaeus, 1758)

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Abstract

Trichiurus lepturus (ribbon fish) was collected from different spatial locations (south west (SW) coast edging the Arabian Sea and south east (SE) coast surrounding the Bay of Bengal of India) and seasons (pre-monsoon, monsoon and post-monsoon) during a continuous period of four years (2008 through 2011), and their edible muscles were studied for differential nutritional compositions of protein, amino acids, vitamins and minerals. The MODIS/ AQUA-derived near-surface chlorophyll-a concentration of its habitats were taken into account to understand their effect on the nutrient signatures of ribbon fish throughout the study period and locations. Seasonal mean protein content attained its maximum during monsoon along the SW (23.4 g/100 g) and SE (11.7 g/100 g) coasts, with higher proportions of essential amino acids (60%) recorded in the samples obtained from the SW coast. The essential to non-essential amino acid ratio was found to be more than 1.0 during the three seasons (> 1.2) along the SW coast, and during monsoon along the SE coast. Total aromatic (TArAA) and total sulphated amino acids (TSAA) recorded monsoon maxima along the SE and SW coasts. Amino acid scores observed monsoon and post-monsoon maxima along the SW and SE coasts, respectively. Mineral content in *T. lepturus* collected from the SE coast was found to be significantly higher during the monsoon season. Significant seasonal variations of vitamin content in *T. lepturus* were observed along the study locations with higher vitamin A, D_3 and C contents at the SW coast and vitamins E and K_1 at the SE coast. The present study demonstrated *T. lepturus* as a valuable source of the well balanced proteins/amino acids with high-biological value, minerals, and vitamins to be qualified as a preferred health food for human diet.

Introduction

Fish muscle is the cheapest source of animal protein with essential amino acids (lysine, methionine, cystine, threonine), minerals (Ca, P, Na etc) and vitamins, especially, fat soluble vitamins, which are required in human diet for good health (Monalisa *et al*., 2013). The protein quality of food depends on their digestibility and content of essential amino acids like leucine, lysine and phenylalanine (Robbins *et al*., 2010). Amino acid composition is one of the most important nutritional qualities of protein and the amino acid score (AAS) (FAO/WHO, 1991) is used to evaluate protein quality world-wide (Iqbal *et al*., 2006). The amino acid composition of any food proteins has significant role in various physiological activities of human body and affects either directly or indirectly in maintaining good health. In general, animal proteins have an amino acid composition that is more favorable than plant proteins, and the protein quality of most fish may exceed that of terrestrial meat and be equal to an ideal protein such as lacto albumin (Friedman, 1996). Therefore, establishment of optimal dietary requirements of amino acids and characterization of alternative protein/amino acid sources have been a major focus of fish nutrition research. Vitamins and minerals are nutrients required in very small amounts for essential metabolic reactions in the body. Fish can transport various minerals and vitamins necessary for good health. Fish fat is a rich source of vitamins, including vitamin A, D, E, K, which must be taken on a regular basis because of their key roles in human health and metabolism (Boran *et al*., 2011). Fish can contribute appreciable amounts of dietary calcium, iron and zinc, nutrients that tend to be low in human diets. Minerals such as iron, manganese and zinc are essential and play important roles in biological systems. Essential minerals like selenium are also abundant in seafood compared to mammalian meat (Larsen *et al*., 2011).

Ribbon fish (hair-tails or cutlass fishes) (*Trichiurus lepturus*; Linnaeus, 1758) is a pelagic fish, which occupy an important place among the food fishes of India. The ribbon fishes belong to the family Trichiuridae and are represented in Indian waters by four species, namely, *Trichiurus lepturus*, *Lepturacanthus savala*, *Eupleurogrammus intermedius* and *E. muticus*. Among these, *T. lepturus*

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is most abundant along the Indian coasts of the Arabian Sea and Bay of Bengal. *T. lepturus* is carnivorous in nature and piscivorous in habit, although very often reported to exhibit cannibalism (Thiagarajan *et al*., 1992). *T. lepturus* spawns more than once in a year and the peak spawning of this species was observed during April to June in SW coast. But in SE coast the species spawns during February to June, with the peak in May. Ribbon fishes occupy an important place among Indian marine fishes judged by the magnitude of the fishery they support as they ranked seventh among the exploited fish group in order of predominance (James *et al*., 1986).

The information concerning the nutritional value of ribbon fish is still scarce, though it is a dominant marine fishery resource off the Indian coast. Therefore, a proper understanding about the biochemical constituents of this species has become a primary requirement for the nutritionists and dieticians. The study of seasonal amino acid, vitamin and mineral content of food fishes like *Trichiurus lepturus* is of importance for conclusions on their properties as a source of the essential components for humans. This study, therefore designed to examine the spatial (south west and south east coasts of India bordering the Arabian Sea and Bay of Bengal, respectively), seasonal (pre-monsoon, monsoon and post-monsoon) and inter-annual (2008 through 2011) variations among the protein, amino acid, vitamin and mineral composition in the edible muscles of *T. lepturus*. The relative abundance of chlorophyll-a concentration derived from SeaWiFS data obtained from MODIS-AQUA data for the studied period were also taken into account to understand their effect on these nutritional parameters throughout the study period.

Materials and Methods

Samples

Fresh ribbon fishes were collected (1 kg each) from fishing harbors of South west coast (Mangalore, Calicut and Cochin) and South east coast (Chennai, Mandapam and Tuticorin) during the period of 2008 to 2011 in three different quarters, viz. pre-monsoon (January – April), monsoon (May – August), and post-monsoon (September – December). The time interval between capturing and the arrival of the fish at the landing sites was about 3-4 hours. The collected samples were kept in ice and transferred to the laboratory. The samples were then washed with chilled water. After taking the weights and lengths of the fish, whole fish were immediately dressed to remove scales, head, and viscera. The edible muscle was separated manually, ground in a mincer and packed in insulated containers at -20^{o}C for further analyses.

True protein and amino acid analysis

The true protein contents of the ribbon fishes were estimated by the established method (Lowry *et al*., 1951). The absorbance of the protein aliquot was measured at 660 nm in a UV-Visible spectrophotometer (Varian Cary, USA) within 15 min against the reagent blank. The true protein content of the sample was calculated from the standard curve of bovine serum albumin, and expressed as g/100 g edible muscle. The amino acid content of the ribbon fishes was measured using the Pico - Tag method as described earlier (Heinrikson and Meredith, 1984) using suitable modifications (Chakraborty *et al*., 2013). The sample was hydrolyzed for 24 h at 110°C with 6 M HCl in sealed glass tubes filled with nitrogen. The hydrolyzed samples were treated with redrying reagent (MeOH 95%: water: triethylamine, 2:2:1 v/v/v), and thereafter pre-column derivatization of hydrolyzable amino acids was performed with phenylisothiocyanate (PITC, or Edman's reagent) to form phenylthiocarbamyl (PTC) amino acids. The reagent was freshly prepared, and the composition of derivatising reagent (methanol 95%: triethylamine: phenylisothiocyanate, 20 µL, 7:1: 1 v/v/v). The derivatized sample (PTC derivative, 20 µL) was diluted with sample diluent (20 µL, 5 mM sodium phosphate NaHPO4 buffer, pH 7.4: acetonitrile 95:5 v/v) before being injected into reversed-phase binary gradient HPLC (Waters reversed-phase PICO. TAG amino acid analysis system), fitted with a packed column (dimethylocatadecylsilyl- bonded amorphous silica; Nova-Pak C18, 3.9 X 150 mm) maintained at 38±1°C in a column oven to be detected by their UV absorbance (λmax 254 nm; Waters 2487 dual absorbance detector). The mobile phase used were eluents A and B, whereas eluent A comprises sodium acetate trihydrate (0.14 M, 940 ml, pH 6.4) containing triethylamine (0.05%), mixed with acetonitrile (60 ml), and eluent B used was acetonitrile : water (60:40, v/v). A gradient elution program, with increasing eluent B was employed for this purpose. An additional step of 100% eluent B is used to wash the column prior to returning to initial conditions. Standard (PIERS amino acid standard H; Thermoscientific) was run before each sample injection. Samples (PTC amino acid derivatives) were injected in triplicate, and the output was analyzed using BREEZE software. The quantification of amino acids was carried out by comparing the sample with the standard, and the results were expressed in g/100 g edible muscle.

Estimation of nutritional indices and amino acid score

The total essential amino acids (TEAA), total non-essential amino acids (TNEAA), total amino acids (TAA), total aromatic amino acids (TArAA), total sulfur containing amino acids (TSAA) and the ratios of total essential amino acid (TEAA) to total non-essential amino acid (TNEAA), i.e. (TEAA/TNEAA); total essential amino acid (EAA) to the total amino acid (TAA), i.e. (TEAA/TAA); total non-essential amino acid (TNEAA) to the total amino acid (TAA), i.e. (TNEAA/TAA), leucine/isoleucine (Leu/ILeu), arginine/lysine (Arg/Lys), cysteine in total sulfur containing amino acids (Cys/TSAA) were calculated. The amino acid score (AS) for the essential amino acids was calculated using the FAO/WHO (FAO/WHO, 1991) formula: amount of amino acid per sample protein (mg/g) /amount of amino acid per protein in reference protein (mg/g)., with respect to reference amino acid requirements for adults (FAO/WHO/UNU, 2007).

Determination of fat soluble vitamins

Estimation of fat soluble vitamins was carried out by the method of Salo-Vaananen *et al.* (2000) with suitable modifications (Chakraborty *et al*., 2013). The stock solutions of vitamin standards (Sigma-Aldrich Chemical Co. Inc, St. Louis, MO) were prepared (1, 10, 25, 50, & 100 ppm) to draw the standard curve by HPLC. All the stock solutions were stored at -20°C except vitamin D_3 where the stock solutions were stored at 4°C. Aliquots of the lipids extracted from the edible muscle were hydrolyzed with KOH/MeOH (0.5N, 2 ml) at 60°C for 30 min to furnish the hydrolyzed mixture, which (2 ml) was thereafter extracted with petroleum ether (12 ml), and washed with distilled water (2 x 8 ml) to make it alkali-free. The non-saponifiable matter (8 ml) was concentrated using a rotary evaporator (Heidolph, Germany; 50°C), reconstituted in MeOH, filtered through nylon acrodisc syringe filter (0.2 µm) to be injected (20 µL) in HPLC (Shimadzu, Prominence) equipped with a C18 column (Phenomenex, 250 mm length, 4.6 mm I.D., 5µm) in column oven (32°C) and connected to a PDA detector. The run time was 45 min, and the eluents were detected at 265 nm using the gradient program as follows: 20% MeOH up to 3 min, which was increased to 100% in the next 5 min and held for 37 min. The flow rate was 1 ml/min. Vitamin C was determined based upon the quantitative discoloration of 2, 6-dichlorophenol indophenol titrimetric method as described (AOAC, 2005). The vitamins A, D3, E, K_1 and C were expressed as µg/100 g edible muscle.

Estimation of minerals

Estimation of minerals was carried out by atomic absorption spectrophotometer (CHEMITO AA 203) following the di-acid ($HNO_3/HClO_4$) digestion method with suitable modifications (Astorga-Espana *et al*., 2007; Chakraborty *et al*., 2013). The analyses of Ca, Na, K, Mn, Fe, and Zn were performed by flame atomic absorption spectrophotometry (AAS) equipped with a hollow cathode lamp containing D2 lamp background correction system. For selenium, continuous flow hydride generator coupled with an atomic absorption spectrometer was used. Phosphorus content was analyzed by an alkalimetric ammonium molybdophosphate method as described in AOAC official method 964.06 (AOAC, 2005).

Chlorophyll-a concentration

Chlorophyll-a concentration derived from the global 9-km monthly mean SeaWiFS (Sea Viewing Wide Field-of-view Sensor) data for the period from January 2008 to December 2011 (Chakraborty *et al*., 2013; Chakraborty *et al*., 2014) were taken into account to indicate the distribution of the photosynthetic pigment chlorophyll-a, and expressed as mg/m^3.

Statistical analyses

Statistical evaluation was carried out with the Statistical Program for Social Sciences 13.0 (SPSS Inc, Chicago, USA, ver. 13.0). Analyses were carried out in triplicate, and the means of all parameters were examined for significance by analysis of variance (ANOVA). Pearson correlation coefficient between the mean values of parameters examined were calculated and the level of significance for all analyses was reported at $p < 0.05$.

Results and Discussion

Seasonal and inter-annual variability in chlorophyll-a concentration

The variance in the spatial distribution of chlorophyll-a during 2008- 2011, with respect to three seasons (pre-monsoon, monsoon and post-monsoon) have been computed in an earlier study (Chakraborty *et al*., 2013), which demonstrated relatively lower values during the pre-monsoon season (four-year pre-monsoon average of 0.3 mg/m^3), reached monsoon maxima (1.2 mg/m^3), and subsequently decreased throughout the post-monsoon season (0.5 mg/m^3). The chlorophyll-a content recorded at its maximum during the monsoon and post-monsoon seasons (0.8 mg/m^3) on the SE coast, and minimum during pre-monsoon period (0.7 mg/m^3) (Chakraborty *et al*.,

Table 1A. Protein (g/100 g edible portion) and amino acid composition (g/100 g edible portion) of *T. lepturus* collected from the south west coast of India during 2008-2011 in three different seasons (pre-monsoon, monsoon and post-monsoon).

	Pre-monsoon				Monsoon				Post-monsoon			
	2008	2009	2010	2011	2008	2009	2010	2011	2008	2009	2010	2011
Protein	10.25±1.10^{a}	11.26±1.11^{a}	11.24±1.16^{a}	8.89±0.96^{a}	23.69±2.47^{b}	24.56±2.37^{b}	23.56±2.18^{b}	22±2.17^{b}	14.02±1.39^{a}	9.25±0.92^{a}	7.69±0.75^{a}	9.65±0.96^{a}
Histidine (His)^{a} (1.9 mg/100 g)	0.19±0.03^{a}	0.23±0.03^{a}	0.27±0.04^{a}	0.10±0.01^{a}	2.08±0.3^{b}	2.06±0.29^{b}	2.07±0.30^{b}	2.04±0.29^{b}	0.53±0.08^{a}	0.30±0.04^{a}	0.07±0.01^{a}	0.19±0.03^{a}
Arginine (Arg)^{a}	0.59±0.08^{a}	0.69±0.10^{a}	0.80±0.11^{a}	0.39±0.06^{a}	2.43±0.35^{b}	2.36±0.34^{b}	2.40±0.34^{b}	2.3±0.33^{b}	0.98±0.14^{a}	0.61±0.09^{a}	0.99±0.04^{a}	0.43±0.06^{a}
Threonine^{a} (Thr)(3.4 mg/100 g)	0.31±0.04^{a}	0.35±0.05^{a}	0.40±0.06^{a}	0.21±0.03^{a}	1.05±0.15^{b}	1.03±0.15^{b}	1.04±0.15^{b}	1.01±0.14^{b}	0.93±0.13^{b}	0.53±0.08^{a}	0.95±0.02^{a}	0.33±0.05^{a}
Valine^{a} (Val)(3.5 mg/100g)	0.40±0.06^{a}	0.45±0.06^{a}	0.50±0.07^{a}	0.31±0.04^{a}	1.36±0.19^{b}	1.32±0.19^{b}	1.34±0.19^{b}	1.28±0.18^{b}	0.66±0.09^{a}	0.42±0.06^{a}	0.20±0.03^{a}	0.31±0.04^{a}
Methionine^{a} (Met)	0.25±0.04^{a}	0.28±0.04^{a}	0.32±0.05^{a}	0.17±0.02^{a}	0.67±0.10^{b}	0.60±0.09^{b}	0.64±0.09^{b}	0.54±0.08^{b}	0.63±0.09^{b}	0.35±0.05^{a}	0.69±0.01^{a}	0.21±0.03^{a}
Isoleucine^{a} (Ileu) (2.8 mg/100 g)	0.39±0.06^{a}	0.42±0.06^{a}	0.45±0.07^{a}	0.32±0.05^{a}	1.01±0.14^{bc}	0.94±0.13^{bc}	0.98±0.14^{bc}	0.88±0.13^{bc}	0.66±0.09^{ac}	0.43±0.06^{a}	0.71±0.03^{a}	0.32±0.05^{a}
Leucine^{a} (Leu)(6.6 mg/100 g)	0.59±0.08^{a}	0.67±0.10^{a}	0.75±0.11^{ac}	0.43±0.06^{a}	1.29±0.18^{bc}	1.34±0.19^{bc}	1.31±0.19^{bc}	1.39±0.20^{bc}	1.09±0.16^{ac}	0.69±0.10^{a}	1.29±0.04^{a}	0.49±0.07^{a}
Phenylalanine^{a} (Phe)	0.33±0.05^{a}	0.38±0.05^{ac}	0.43±0.06^{ac}	0.23±0.03^{a}	0.99±0.14^{bc}	0.93±0.13^{bc}	0.96±0.14^{bc}	0.87±0.12^{bc}	0.73±0.10^{bc}	0.45±0.06^{ac}	0.18±0.03^{a}	0.31±0.04^{a}
Lysine^{a} (Lys)(5.8 mg/100 g)	0.78±0.11^{a}	0.89±0.13^{a}	0.99±0.15^{a}	0.57±0.06^{a}	1.40±0.15^{a}	1.34±0.15^{a}	1.37±0.15^{a}	1.28±0.14^{a}	1.35±0.15^{a}	0.83±0.10^{a}	0.32±0.04^{a}	0.58±0.06^{a}
Alanine (Ala)^{b}	0.52±0.07^{a}	0.57±0.08^{a}	0.63±0.09^{a}	0.41±0.06^{a}	1.61±0.23^{b}	1.56±0.22^{b}	1.58±0.23^{b}	1.50±0.22^{b}	0.65±0.09^{a}	0.46±0.07^{a}	0.28±0.04^{a}	0.37±0.05^{a}
Cysteine (Cys)^{b}	0.04±0.01^{a}	0.06±0.01^{a}	0.08±0.01^{a}	0.01±0.01^{a}	1.11±0.30^{b}	1.01±0.29^{b}	1.06±0.29^{b}	0.92±0.27^{b}	0.31±0.04^{a}	0.15±0.02^{a}	0.16±0.01^{a}	0.08±0.01^{a}
Glutamic acid (Glu)^{b}	1.25±0.18^{a}	1.45±0.21^{a}	1.64±0.23^{a}	0.87±0.12^{a}	1.01±0.57^{b}	0.78±0.54^{b}	0.89±0.56^{b}	0.55±0.51^{b}	2.16±0.31^{a}	1.34±0.19^{a}	1.25±0.07^{a}	0.93±0.13^{a}
Glycine (Gly)^{b}	0.32±0.05^{ac}	0.37±0.05^{ac}	0.41±0.06^{ac}	0.23±0.03^{a}	0.58±0.08^{ac}	0.61±0.09^{bc}	0.59±0.08^{bc}	0.64±0.09^{bc}	0.53±0.08^{ac}	0.34±0.05^{ac}	0.15±0.02^{a}	0.25±0.04^{a}
Proline (Pro)^{b}	0.33±0.05^{a}	0.39±0.06^{a}	0.45±0.06^{a}	0.21±0.03^{a}	1.61±0.23^{b}	1.56±0.22^{b}	1.58±0.23^{b}	1.51±0.22^{b}	0.45±0.06^{a}	0.31±0.04^{a}	0.16±0.02^{a}	0.24±0.03^{a}
Serine (Ser)^{b}	0.27±0.04^{a}	0.31±0.04^{a}	0.34±0.05^{a}	0.2±0.03^{a}	0.67±0.10^{bc}	0.71±0.1^{bc}	0.69±0.10^{bc}	0.74±0.11^{bc}	0.68±0.10^{bc}	0.40±0.06^{ac}	0.45±0.02^{a}	0.27±0.04^{a}
Tyrosine (Tyr)^{b}	0.17±0.02^{a}	0.19±0.03^{a}	0.22±0.03^{ac}	0.13±0.02^{a}	0.37±0.05^{a}	0.4±0.06^{b}	0.39±0.06^{b}	0.43±0.06^{b}	0.42±0.06^{b}	0.26±0.04^{b}	0.69±0.01^{b}	0.16±0.02^{a}
TEAA	3.83±0.35^{ac}	4.36±0.55^{ac}	4.91±0.46^{ac}	2.73±0.3^{a}	12.28±1.38^{ac}	11.92±1.09^{bc}	12.11±1.28^{bc}	11.59±1.19^{bc}	7.56±0.78^{ac}	4.61±0.5^{ac}	5.40±0.50^{ac}	3.17±0.40^{a}
TNEAA	2.90±0.25^{ac}	3.34±0.35^{ac}	3.77±0.36^{ac}	2.06±0.23^{a}	6.96±0.78^{ac}	6.63±0.69^{bc}	6.78±0.68^{bc}	6.29±0.63^{bc}	5.20±0.58^{ac}	3.26±0.35^{ac}	3.14±0.32^{a}	2.30±0.24^{a}
TAA	6.73±0.65^{ac}	7.70±0.75^{ac}	8.68±0.96^{ac}	4.79±0.53^{a}	19.24±2.08^{ac}	18.55±1.85^{bc}	18.89±1.95^{bc}	17.88±1.79^{bc}	12.76±1.28^{ac}	7.87±0.75^{ac}	8.54±0.82^{a}	5.47±0.54^{a}

[a]Essential amino acids; [b]Non-essential amino acids

TEAA- Total amino acids; TNEAA – Total non-essential amino acids; TAA - Total amino acids

Data are expressed as mean ± standard deviation (n = 3);

Pre-monsoon: February to May; monsoon: June to September; post-monsoon: October to January;

Different superscripts (a-c) within a row denote significant differences ($p < 0.05$). FAO/WHO reference pattern (1985) for evaluating proteins (mg/ 100 g) were indicated in parentheses (FAO/WHO, 1985)

Tryptophan was not determined.

Table 1B. Protein (g/100 g edible portion) and amino acid composition (g/100 g edible portion) of *T. lepturus* collected from the south east coast of India during 2008-2011 in three different seasons (pre-monsoon, monsoon and post-monsoon).

	Pre-monsoon				Monsoon				Post-monsoon			
	2008	2009	2010	2011	2008	2009	2010	2011	2008	2009	2010	2011
Protein	12.05±1.29^{a}	11.02±1.2^{a}	8.56±0.99^{a}	7.13±0.71^{a}	11.56±1.25^{a}	10.25±1.17^{a}	10.69±1.08^{a}	14.56±1.58^{b}	9.69±1.25^{a}	9.56±0.94^{a}	10.25±1.23^{a}	11.02±1.41^{a}
Histidine (His)^{a}	0.44±0.08^{a}	0.55±0.08^{b}	0.19±0.03^{b}	0.11±0.02^{b}	0.36±0.05^{b}	0.22±0.03^{b}	0.24±0.05^{a}	0.69±0.07^{b}	0.27±0.04^{b}	0.29±0.04^{b}	0.30±0.04^{b}	0.62±0.09^{b}
Arginine(Arg)^{a}	0.42±0.09^{a}	0.87±0.12^{b}	0.70±0.10^{b}	0.41±0.06^{b}	0.16±0.02^{b}	0.12±0.02^{b}	0.15±0.09^{c}	0.12±0.03^{a}	0.25±0.04^{b}	0.22±0.03^{b}	0.18±0.03^{b}	0.11±0.07^{a}
Threonine^{a}(Thr)	0.28±0.05^{a}	0.32±0.05^{b}	0.32±0.05^{b}	0.22±0.03^{b}	0.29±0.04^{b}	0.14±0.02^{b}	0.14±0.08^{c}	0.56±0.06^{a}	0.48±0.07^{b}	0.46±0.07^{b}	0.43±0.06^{b}	0.35±0.18^{a}
Valine^{a}(Val)	0.29±0.04^{a}	0.39±0.06^{b}	0.37±0.05^{b}	0.30±0.04^{b}	0.08±0.01^{b}	0.08±0.01^{b}	0.09±0.10^{c}	0.09±0.01^{a}	0.16±0.02^{b}	0.15±0.02^{b}	0.14±0.02^{b}	0.11±0.15^{a}
Methionine^{a}(Met)	0.66±0.09^{a}	0.42±0.06^{a}	0.20±0.03^{a}	0.11±0.02^{a}	0.42±0.06^{a}	0.25±0.04^{a}	0.35±0.22^{b}	0.88±0.13^{a}	0.74±0.11^{a}	0.69±0.10^{a}	0.64±0.09^{a}	0.79±0.11^{a}
Isoleucine^{a}(Ileu)	0.97±0.14^{a}	0.41±0.06^{a}	0.35±0.05^{a}	0.31±0.04^{a}	0.04±0.01^{b}	0.01±0.00^{b}	0.38±0.28^{c}	0.99±0.14^{a}	0.09±0.01^{b*}	0.09±0.01^{b}	0.08±0.01^{b}	0.89±0.13^{a}
Leucine^{a}(Leu)	0.35±0.24^{a}	0.45±0.06^{b}	0.60±0.09^{b}	0.42±0.06^{b}	0.30±0.04^{b}	0.18±0.03^{b}	0.14±0.51^{c}	0.85±0.27^{a}	0.48±0.07^{b}	0.43±0.06^{b}	0.38±0.05^{b}	0.36±0.24^{a}
Phenylalanine^{a}(Phe)	0.87±0.12^{a}	0.54±0.08^{a}	0.30±0.04^{a}	0.22±0.03^{a}	0.63±0.09^{a}	0.36±0.05^{a}	0.19±0.25^{b}	1.04±0.15^{a}	0.99±0.14^{a}	0.92±0.13^{a}	0.83±0.12^{a}	0.94±0.13^{a}
Lysine^{a}(Lys)	0.78±0.11^{a}	0.57±0.08^{a}	0.78±0.11^{a}	0.55±0.08^{a}	0.19±0.03^{a}	0.10±0.01^{a}	0.11±0.65^{c}	0.12±0.33^{b}	0.16±0.02^{a*}	0.21±0.03^{a}	0.24±0.03^{a}	0.32±0.30^{b}
Alanine(Ala)^{b}	0.69±0.14^{a}	0.33±0.05^{b}	0.47±0.07^{b}	0.35±0.05^{b}	0.19±0.03^{b}	1.56±0.01^{b}	1.85±0.27^{c}	0.97±0.14^{a}	0.30±0.04^{b}	0.29±0.04^{b}	0.27±0.04^{b}	0.87±0.12^{a}
Cysteine(Cys)^{b}	1.24±0.30^{a}	0.48±0.07^{b}	0.04±0.01^{b}	0.09±0.01^{b}	0.13±0.02^{b}	0.54±0.01^{b}	0.48±0.07^{b}	0.28±0.04^{b}	0.2±0.030^{b}	0.19±0.03^{b}	0.18±0.03^{b}	0.25±0.04^{b}
Glutamic acid (Glu)^{b}	1.54±0.53^{a}	0.50±0.07^{b}	1.26±0.18^{b}	0.85±0.12^{b}	0.83±0.12^{b}	0.44±0.06^{b}	0.54±1.23^{c}	0.65±0.65^{a}	1.39±0.20^{b}	1.34±0.19^{b}	1.29±0.18^{b}	1.03±0.58^{a}
Glycine(Gly)^{b}	0.84±0.12^{a}	0.24±0.03^{b}	0.27±0.04^{b}	0.20±0.03^{b}	0.14±0.02^{b}	1.45±0.01^{b}	1.41±0.20^{c}	0.74±0.11^{a}	0.36±0.05^{b}	0.29±0.04^{b}	0.22±0.03^{b}	0.67±0.10^{a}
Proline(Pro)^{b}	0.25±0.15^{a}	0.39±0.06^{b}	0.34±0.05^{b}	0.15±0.02^{b}	0.41±0.06^{b}	0.30±0.04^{b}	1.10±0.31^{c}	0.24±0.18^{a}	0.50±0.07^{b}	0.49±0.07^{b}	0.47±0.07^{b}	0.41±0.16^{a}
Serine(Ser)^{b}	0.54±0.08^{a}	0.38±0.05^{a}	0.25±0.04^{a}	0.23±0.03^{a}	0.79±0.11^{a}	0.4±0.06^{a}	0.98±0.14^{b}	0.69±0.10^{a}	0.28±0.04^{a}	0.72±0.10^{a}	1.16±0.17^{b}	0.62±0.09^{a}
Tyrosine(Tyr)^{b}	0.27±0.04^{a}	0.43±0.06^{a}	0.16±0.02^{a}	0.09±0.01^{a}	3.14±0.45^{b}	2.18±0.31^{c}	0.58±0.08^{a}	1.38±0.20^{c}	0.36±0.05^{a}	0.33±0.05^{a}	0.29±0.04^{a}	0.24±0.18^{c}
TEAA	5.06±0.51^{a}	4.52±0.48^{a}	3.81±0.11^{a}	2.65±0.28^{a}	2.47±0.23^{a}	1.46±0.11^{a}	1.79±0.15^{c}	5.34±0.63^{b}	3.62±0.30^{a}	3.46±0.30^{a}	3.22±0.30^{a}	4.49±0.30^{b}
TNEAA	5.37±0.08^{a}	2.75±0.05^{a}	2.79±0.04^{a}	1.96±0.03^{a}	5.63±0.11^{a}	6.87±0.06^{a}	6.94±0.14^{b}	4.95±0.10^{a}	3.39±0.04^{a}	3.65±0.10^{a}	3.88±0.17^{b}	4.09±0.09^{a}
TAA	10.43±1.04^{a}	7.27±0.76^{a}	6.60±0.62^{a}	4.61±0.41^{a}	8.10±0.85^{a}	8.33±0.81^{a}	8.73±0.90^{a}	10.29±1.20^{a}	7.01±0.75^{a}	7.11±0.75^{a}	7.10±0.74^{a}	8.58±0.86^{a}

Data are expressed as mean ± standard deviation (n = 3)

Different superscripts (a-c) within a row denote significant differences ($p < 0.05$). Other notations are as indicated in Table 1A.

2013).

Inter-annual and seasonal variability of true protein content in Trichiurus lepturus

The true protein content in the edible muscles of *T. lepturus* collected from the SW and SE coasts are shown in Table 1A and 1B, respectively. Proteins are an essential nutritional component in *T. lepturus*, and are essentially required by the human beings for growth and survival. The true protein content found to be 7.6-24.6 g/100 g in the SW coast and 7.1-14.6 g/100 g in the SE coast samples and the mean seasonal value observed monsoon maxima along both SW and SE coasts (four year mean of 23.4 and 11.7 g/100 g, respectively). The true protein content of ribbon fishes was comparatively higher than maize (8.0-11.0 g/100 g), wheat (11.0-14.0 g/100 g), rice (7.0-9.0 g/100 g), barley (8.0-11.0 g/100 g), oats (12.0-14.0 g/100 g) and sorghum (9.0-11.0 g/100 g). The significantly higher protein content during monsoon may be due to the higher intake of food in this season as it correlated well with high chlorophyll-a content in monsoon. Apparently, during the monsoon season, the upwelling of deeper water brings the nutrient-laden water closer to the surface. The low protein content was observed during post-monsoon along SW coast and pre-monsoon along SE coast (four year mean of 10.2 and 9.7 g/100 g, respectively). The low protein content in post-monsoon within both coasts may be due to the fact that amino acid related to depletion select materials for building up of the latent gonads in this season (Jan *et al*., 2012). In general, the seasonal profile of the true protein from ribbon fishes collected from both the coasts exemplified the active growing phase during monsoon and a decrease after spawning, in post-monsoon.

Inter-annual and seasonal variability of amino acids in Trichiurus lepturus

The essential and non-essential amino acid

Table 2. Nutritional indices and essential amino acid scores (%) of *T. lepturus* collected from the south west and south east coasts of India during 2008-2011 in three different seasons.

	Pre-monsoon				Monsoon				Post-monsoon			
	2008	2009	2010	2011	2008	2009	2010	2011	2008	2009	2010	2011
SOUTH WEST COAST												
∑EAA/∑NEAA	1.32±0.05^{ac}	1.31±0.05^{ac}	1.30±0.06^{ac}	1.33±0.03^{a}	1.76±0.08^{ac}	1.8±0.09^{bc}	1.79±0.08^{bc}	1.84±0.09^{bc}	1.45±0.08^{ac}	1.41±0.05^{ac}	1.72±0.02^{a}	1.38±0.04^{a}
∑ArAA	0.69±0.05^{a}	0.80±0.06^{a}	0.92±0.06^{a}	0.46±0.03^{a}	3.44±0.23^{b}	3.39±0.22^{b}	3.42±0.23^{b}	3.34±0.22^{b}	1.68±0.06^{a}	1.01±0.04^{a}	0.94±0.02^{a}	0.66±0.03^{a}
∑SAA	0.29±0.04^{a}	0.34±0.04^{a}	0.40±0.05^{a}	0.18±0.03^{a}	1.78±0.10^{bc}	1.61±0.10^{bc}	1.70±0.10^{bc}	1.46±0.11^{bc}	0.94±0.10^{bc}	0.50±0.06^{ac}	0.85±0.02^{a}	0.29±0.04^{a}
Leu: Ileu	1.51±0.02^{a}	1.60±0.03^{a}	1.67±0.03^{ac}	1.34±0.02^{a}	1.28±0.05	1.43±0.06	1.34±0.06	1.58±0.06	1.65±0.06	1.60±0.04	1.82±0.01	1.53±0.02
Cys: ∑SAA	0.14±0.05^{a}	0.18±0.06^{a}	0.20±0.06^{a}	0.06±0.03^{a}	0.62±0.23^{b}	0.63±0.22^{b}	0.62±0.23^{b}	0.63±0.22^{b}	0.33±0.06^{a}	0.30±0.04^{a}	0.19±0.02^{a}	0.28±0.03^{a}
∑EAA/∑TAA	0.57±0.04^{a}	0.57±0.04^{a}	0.57±0.05^{a}	0.57±0.03^{a}	0.64±0.10^{bc}	0.64±0.10^{bc}	0.64±0.10^{bc}	0.65±0.11^{bc}	0.59±0.10^{bc}	0.59±0.06^{ac}	0.78±0.02^{a}	0.58±0.04^{a}
∑NEAA/∑TAA	0.43±0.02^{a}	0.43±0.03^{a}	0.43±0.03^{ac}	0.43±0.02^{a}	0.36±0.05^{a}	0.36±0.06^{a}	0.36±0.06^{a}	0.35±0.06^{a}	0.41±0.06^{a}	0.41±0.04^{a}	0.46±0.01^{a}	0.42±0.02^{a}
His	98	108	126	59	462	441	462	488	199	171	48	104
Thr	89	91	105	69	130	123	130	135	195	169	363	101
Val	112	114	127	100	164	154	163	166	135	130	74	92
Met + Cys	113	121	142	81	301	262	289	265	268	216	442	120
Ile	136	133	143	129	152	137	149	143	168	166	330	118
Leu	87	90	101	73	83	83	84	96	118	113	254	77
Phe + Tyr	77	80	92	64	91	86	91	94	130	122	180	77
Lys	131	136	152	111	102	94	100	100	166	155	72	104
SOUTH EAST COAST												
∑EAA/∑NEAA	0.94±0.56^{ab}	1.64±0.56^{ab}	1.37±0.56^{ab}	1.35±0.56^{ab}	0.44±0.56^{ab}	0.21±0.56^{ab}	0.26±0.56^{ab}	1.07±0.56^{ab}	1.07±0.56^{ab}	0.95±0.56^{ab}	0.83±0.56^{ab}	1.10±0.56^{ab}
∑ArAA	1.58±0.24^{a}	1.52±0.06^{b}	0.65±0.09^{b}	0.42±0.06^{b}	4.13±0.04^{b}	2.76±0.03^{b}	1.01±0.51^{c}	3.11±0.27^{a}	1.62±0.07^{b}	1.54±0.06^{b}	1.42±0.05^{b}	1.80±0.24^{a}
∑SAA	1.90±0.11^{a}	0.90±0.08^{a}	0.24±0.11^{a}	0.20±0.08^{a}	0.55±0.03^{a}	0.79±0.01^{a}	0.83±0.65^{c}	1.16±0.33^{b}	0.94±0.02^{a}	0.88±0.03^{a}	0.82±0.03^{a}	1.04±0.30^{b}
Leu: Ileu	0.36±0.11^{a}	1.10±0.08^{a}	1.71±0.11^{a}	1.35±0.08^{a}	7.50±0.03^{a}	18.0±0.01^{a}	0.37±0.65^{c}	1.87±0.33^{b}	5.33±0.02^{a}	4.78±0.03^{a}	4.75±0.03^{a}	0.40±0.30^{b}
∑EAA/∑AA	0.49±0.14^{a}	0.62±0.05^{b}	0.58±0.07^{bb}	0.57±0.05^{b}	0.30±0.03^{b}	0.18±0.01^{b}	0.21±0.27^{c}	0.52±0.14^{a}	0.52±0.04^{b}	0.49±0.04^{b}	0.45±0.04^{b}	0.52±0.12^{a}
∑NEAA/∑AA	0.51±0.27^{a}	0.38±0.27^{a}	0.42±0.27^{a}	0.43±0.27^{a}	0.70±0.27^{a}	0.82±0.27^{a}	0.79±0.27^{a}	0.48±0.27^{a}	0.48±0.27^{a}	0.51±0.27^{a}	0.55±0.27^{a}	0.48±0.27^{a}
Cys:∑SAA	0.65±0.19^{a}	0.53±0.19^{a}	0.17±0.19^{a}	0.45±0.19^{a}	0.24±0.19^{a}	0.68±0.19^{a}	0.58±0.19^{a}	0.24±0.19^{a}	0.21±0.19^{a}	0.22±0.19^{a}	0.22±0.19^{a}	0.24±0.19^{a}
His	192	263	117	81	164	113	118	249	147	160	154	296
Thr	68	85	110	91	74	40	39	113	146	142	123	93
Val	69	101	124	120	20	22	24	18	47	45	39	29
Met + Cys	631	327	112	112	190	308	311	319	388	368	320	378
Ile	287	133	146	155	12	3	127	243	33	34	28	288
Leu	44	62	106	89	39	27	20	193	75	68	56	50
Phe + Tyr	150	140	85	69	518	393	114	264	221	208	173	170
Lys	112	89	157	133	28	17	18	14	28	38	40	50

Data are expressed as mean ± standard deviation of three replicates; [a]EAA essential amino acids; NEAA nonessential amino acids; TAA Total amino acids; SAA sulfur containing amino acids; ArAA Aromatic amino acids; Means with different superscripts (a,b,c) in the same row indicate statistical difference ($p < 0.05$). Other notations are as indicated in Table 1A.

compositions of *T. lepturus* from the SW and SE coasts are recorded in Table 1A and 1B, respectively. No significant inter-annual differences were observed in the amino acid composition between the samples collected from SW and SE coasts over the studied period (2008 - 2011) ($p > 0.05$). The edible muscles of ribbon fishes, especially from the SW coast, showed significantly higher concentration of essential amino acids when compared with the reference pattern (FAO/ WHO, 1985). Along the SW coast, total amino acid (TAA) was significantly higher ($p < 0.05$) during the monsoon (four year mean of 18.6 g/100 g) compared with pre-monsoon and post-monsoon seasons. However, no significant seasonal variation in TAA content was observed along SE coast. The TAA content observed a significant increment from pre-monsoon to monsoon in both coasts, mainly due to an increase in the glutamic acid, phenyl alanine and serine content. The increase in TAA during monsoon season was in accordance with the total protein content. The most abundant EAA found in the samples from SW coast was lysine during pre-monsoon (four year mean of 0.8 g/100 g), arginine during monsoon (four year mean of 2.4 g/100 g) and leucine during post-monsoon (four year mean of 0.9 g/100 g). However, arginine was the most abundant EAA found on the SE coast during pre-monsoon (four year mean of 0.6 g/100 g), lysine (four year mean of 0.7 g/100 g), during monsoon and phenylalanine (four year mean of 0.9 g/100 g), during post-monsoon. During monsoon, four years mean EAA content was significantly higher along the SW coast (12 g/100 g) ($p < 0.05$). A similar trend was observed along the SE coast. Lysine, which observed pre-monsoon and monsoon maxima along the SW and SE coasts, respectively, is an important precursor for the de novo synthesis of glutamate, the most significant neurotransmitter in the mammalian central nervous system (Papes *et al*., 2001). Ribbon fishes collected from the SW coast possess high lysine content than the SE coast samples which is severely restricted in cereals, the most important staple food in the world. Arginine, one of the most versatile amino acid, which serve as the precursor for the synthesis of protein, nitric oxide (NO), urea, polyamines, proline, glutamate, creatine and agmatine in terrestrial animals, was found maximum during monsoon in the SW coast (Wu and Morris, 1998). However, leucine that observed post-monsoon maximum in the SW coast, promotes the healing of bones, skin and muscle tissue. The high arginine content in the ribbon fish muscles along the SE coast during pre-monsoon enriches its sweet taste with complexity and fullness and yields seafood like flavor. Correspondingly, arginine helps to improve blood flow in the arteries of the heart. Phenylalanine was found to be higher during post-monsoon in the SE coast. This amino acid is transformed into norepinephrine in the body through a variety of metabolic steps, as well as to other active chemicals, such as epinephrine, dopamine, and tyramine, which are important for a good mood and have anti-burnout and anti-depressant properties. No significant inter-annual variations were observed in total non-essential amino acid content (TNEAA) in the studied locations ($p > 0.05$). The NEAA observed a monsoon maxima along the SE and SW coasts (6.7 and 6.1 g/100 g, respectively) ($p < 0.05$). Glutamic acid was most copious NEAA in the ribbon fishes, especially during the pre and post-monsoon seasons along the studied locations. Glutamine is the most abundant free amino acid in the body, comprising nearly 60% of the free intracellular amino acids in skeletal muscle. The efflux of glutamine from muscle in critical illness serves as an important carrier of ammonia (nitrogen) to the splanchnic area and the immune system (Deutz *et al.*, 1992). As a donor of nitrogen in the synthesis of purines and pyrimidines, glutamine is essential for the proliferation of cells. However, alanine was the most abundant NEAA found during monsoon season along the SE and SW coasts, and plays a significant role in several metabolic processes and in regulating blood sugar.

Inter-annual and seasonal variability of amino acid based nutritional indices in Trichiurus lepturus

The nutritional indices with respect to different amino acid ratios of *T. lepturus* collected from the SW and SE coasts of India are shown in Table 2. The ratio of essential amino acids (EAA) to non-essential amino acids (NEAA) ranged between 1.8 – 1.3 & 1.6 – 0.4 along the SW and SE coasts, respectively. EAA/NEAA ratio observed monsoon maximum along the SW coast, and pre-monsoon peak along the SE coast (four year mean of 1.8 & 1.3 g/100 g edible muscle, respectively). The EAA / NEAA ratio, which observed more than 1.0 during all the seasons along SW coast and pre-monsoon/post-monsoon off SE coast (> 1.6) samples, indicated that ribbon fishes in these seasons could provide high quality proteins or well-balanced protein deposition. Any ratio of EAA/NEAA amino acids, higher than 1.0 is considered to be ideal, and therefore it can be concluded that ribbon fishes from both coasts, especially SW coast are sources of well balanced and high-quality protein source. The EAA/NEAA ratio observed by Iwasaki and Harada (Iwasaki and Harada, 1985) were considerably lower for other marine species like *Pagrus major* (0.77), *Scomber japonicus* (0.77), *Oncorhynchus keta* and *Paralichthys olivaceus* (0.77), while compared to the present study. The EAA/TAA ratio observed pre-monsoon maximum along SE coast (four year mean of 0.6). However, the EAA/TAA ratios in the edible muscle of ribbon fishes were higher than 50%, which are well above the 39% considered to be adequate for ideal protein food for infants, 26% for children and 11% for adults (FAO, 1985). Exceptionally, the samples collected during the monsoon season along the SE coast showed significantly lower EAA/TAA ratio (0.3). Apparently, high NEAA/TAA ratio was found to be monsoon maxima along the SE coast (four year mean of 0.7). The amount of total aromatic amino acids (TArAA) was recorded to be higher during the monsoon. Correspondingly, leucine: isoleucine (Leu:Ileu) ratio showed best values in post-monsoon along SW coast (four year mean of 1.65) and monsoon along SE coast (four year mean of 7.0). Leu:Ileu ratios of the ribbon fishes from the SE and SW coasts were typical of the ideal ratio suggested by FAO/ WHO (FAO/WHO/UNU, 2007). Deosthale *et al.* (1970) showed that excess leucine in foods interferes with the utilization of isoleucine and lysine. The TSAA showed higher values in monsoon along the SW coast (1.6 g/100 g) ($p < 0.05$), whereas no significant difference were observed in the TSAA content along the SE coast over the three seasons ($p > 0.05$). The sulfur-containing amino acid, methionine cannot be synthesized de novo in humans. Likewise, cysteine can be made from homocysteine but cannot be synthesized on its own. Cys: TSAA ratio showed higher values during the monsoon season along the SW coast (0.6). The amino acid scores (with respect to His, Thr, Val, Met+Lys, Ile, Leu, Phe+Tyr and Lys) of *T. lepturus* collected from the SW and SE coasts of India are shown in Table 2. The amino acid scores were found to be higher during the monsoon with respect to His (four year mean of 463), TSAA (four year mean of 279) along the SW coast, and Phe+Tyr (four year mean of 322). However, during the post-monsoon season, the amino acid scores with respect to Thr, Ileu, Leu and Phe+Tyr were found to be higher in the ribbon fishes along the SW coast, whereas His and TSAA were at their maxima in the samples collected from the SE coast. The amino acid score is indicative of the maximum percentage of protein that may be retained for growth, and these results coincide with the hypothesis proposed by Garcia and Valverde (2006.).

Inter-annual and seasonal variability of vitamin content in Trichiurus lepturus

Ribbon fish lipid is a rich source of fat soluble vitamins, including A, D_3, E and K_1, which must be taken on a regular basis because of their key roles in human health and metabolism. The vitamin content of *T. lepturus* collected from SW and SE coast of India is shown in Table 3. No significant differences ($p > 0.05$) in fat soluble vitamin A, D_3, E and K_1 content were observed between the samples collected from the SW and SE coasts over four years (2008 - 2011). The spatio-seasonal disparity observed in these vitamin levels could be the result of the season, life stage, age or availability of nutrition in the ocean. The seasonal mean vitamin A content in the muscles of ribbon fishes was significantly higher during the post-monsoon season along the SW coast (8.9 µg/100 g), and SE coasts (2.5 µg/100 g). Vitamin A is important for growth and development, for the maintenance of the immune system and good vision. Generally,

Table 3. Vitamin compositions (µg/100 g edible portion) of *T. lepturus* collected from the south west and south east coasts of India during 2008-2011 in three different seasons (pre-monsoon, monsoon & post-monsoon).

	Year	Vitamin A	Vitamin D_3	Vitamin E	Vitamin K_1	Vitamin C
				SOUTH WEST COAST		
Pre-monsoon	2008	1.01 ± 0.14^{a}	396.04 ± 56.64^{a}	0.61 ± 0.09^{ac}	0.08 ± 0.01^{a}	12.96 ± 1.85^{a}
	2009	1.01 ± 0.14^{a}	393.72 ± 56.31^{a}	0.61 ± 0.09^{ac}	0.08 ± 0.01^{a}	12.96 ± 1.85^{a}
	2010	1.01 ± 0.14^{a}	391.4 ± 55.98^{a}	0.61 ± 0.09^{ac}	0.08 ± 0.01^{a}	12.96 ± 1.85^{a}
	2011	1.01 ± 0.14^{a}	400.67 ± 57.3^{a}	0.61 ± 0.09^{ac}	0.08 ± 0.01^{a}	12.96 ± 1.85^{a}
Monsoon	2008	3.67 ± 0.52^{a}	525.3 ± 75.13^{a}	0.64 ± 0.09^{ac}	0.41 ± 0.06^{a}	9.81 ± 1.40^{a}
	2009	3.39 ± 0.48^{a}	475.09 ± 67.95^{a}	0.61 ± 0.09^{ac}	0.39 ± 0.06^{a}	9.21 ± 1.32^{a}
	2010	3.53 ± 0.50^{a}	500.19 ± 71.54^{a}	0.62 ± 0.09^{ac}	0.40 ± 0.06^{a}	9.51 ± 1.36^{a}
	2011	3.11 ± 0.44^{a}	424.88 ± 60.77^{a}	0.58 ± 0.08^{ac}	0.36 ± 0.05^{a}	8.61 ± 1.23^{a}
Post-monsoon	2008	8.30 ± 0.12^{b}	261.21 ± 37.36^{ab}	0.27 ± 0.04^{bd}	3.98 ± 0.57^{b}	10.84 ± 1.55^{a}
	2009	8.29 ± 1.19^{b}	393.77 ± 56.32^{a}	0.32 ± 0.05^{ad}	3.98 ± 0.57^{b}	10.06 ± 1.44^{a}
	2010	9.79 ± 1.40^{b}	526.33 ± 75.28^{ac}	0.37 ± 0.05^{acd}	3.74 ± 0.53^{b}	9.29 ± 1.33^{a}
	2011	9.04 ± 1.29^{b}	460.05 ± 65.8^{a}	0.35 ± 0.05^{acd}	3.8 ± 0.54^{b}	9.68 ± 1.38^{a}
				SOUTH EAST COAST		
Pre-monsoon	2008	2.73 ± 0.39^{a}	336.32 ± 48.1^{ac}	0.97 ± 0.14^{ac}	1.01 ± 0.14^{ac}	10.88 ± 1.56^{a}
	2009	2.94 ± 0.42^{a}	232.06 ± 33.19^{ac}	0.63 ± 0.09^{ac}	1.01 ± 0.14^{ac}	9.97 ± 1.43^{a}
	2010	1.01 ± 0.14^{b}	402.73 ± 57.6^{a}	0.61 ± 0.09^{bc}	0.08 ± 0.01^{bc}	12.96 ± 1.85^{a}
	2011	3.07 ± 0.44^{a}	94.76 ± 13.55^{bc}	0.55 ± 0.08^{bc}	1.05 ± 0.15^{ac}	7.89 ± 1.13^{ac}
Monsoon	2008	1.29 ± 0.18^{b}	228.66 ± 32.7^{ac}	0.64 ± 0.09^{ac}	0.43 ± 0.06^{ac}	4.04 ± 0.58^{bc}
	2009	1.01 ± 0.14^{b}	94.76 ± 13.55^{bc}	0.55 ± 0.08^{bc}	0.40 ± 0.06^{ac}	7.89 ± 1.13^{ac}
	2010	1.05 ± 0.15^{b}	510.88 ± 73.07^{b}	0.24 ± 0.03^{b}	0.36 ± 0.05^{ac}	11.54 ± 1.65^{a}
	2011	1.03 ± 0.15^{b}	302.82 ± 43.31^{ac}	0.39 ± 0.06^{bc}	0.38 ± 0.05^{ac}	9.71 ± 1.39^{a}
Post-monsoon	2008	3.10 ± 0.44^{a}	89.51 ± 12.8^{bc}	0.77 ± 0.11^{c}	3.25 ± 0.47^{b}	8.50 ± 1.22^{ac}
	2009	3.04 ± 0.44^{a}	92.13 ± 13.18^{bc}	0.66 ± 0.09^{ac}	2.73 ± 0.39^{b}	8.19 ± 1.17^{ac}
	2010	2.99 ± 0.43^{a}	94.76 ± 13.55^{bc}	0.55 ± 0.08^{bc}	2.21 ± 0.32^{d}	7.89 ± 1.13^{ac}
	2011	0.93 ± 0.13^{b}	272.54 ± 38.98^{ac}	0.35 ± 0.05^{bc}	0.34 ± 0.05^{ac}	8.74 ± 1.25^{ac}

Vitamin A, D_3, E, K_1 and C are represented in µg/100 g edible portion. Data are expressed as mean ± standard deviation (n = 3); Different superscripts (a-c) within a column denote significant differences ($p < 0.05$).

the flesh of the lean fish contains 7.5 to 15 µg/100 g of vitamin A (25 to 50 IU) (Gulsun et al., 2009). Correspondingly, ribbon fish muscles evaluated in the present study contained lower vitamin A content as compared to that reported earlier (Gulsun *et al.*, 2009). Vitamin D_3 content in the ribbon fishes collected from the SW coast was significantly higher during the monsoon season (481 µg/100 g; $p < 0.05$) while for the samples collected from the SE coast, a monsoon maxima (284.5 µg/100 g) was observed. The present study revealed that the edible portion of the ribbon fish as a wealthy source of vitamin D_3, which is essential for the maintenance of normal blood levels of calcium and phosphate (Trivedi *et al.*, 2003). Pre-monsoon/monsoon maxima in the vitamin E content was observed in the ribbon fishes collected from the SW coast (0.61 µg/100 g) while a pre-monsoon maxima in the SE coast samples (0.69 µg/100 g) were monitored. The vitamin E content in the edible muscles of ribbon fish were found to be low (0.2 to 1.0 µg/100 g), which may be attributed to be due to the lower fat level in this species. Vitamin E too acts as an antioxidant molecule against the peroxidation of fatty acid contained in the cellular and sub cellular membrane phospholipids leading to the formation of phenoxyl free radicals. Apparently, vitamin C content was observed to be higher during the pre-monsoon season in the SW and SE coasts (13 and 10.4 µg/100 g, respectively). Vitamin C and E are potent free radical scavengers. Vitamin C is an essential nutrient for humans, but an additional external dietary source is required because it is not synthesized by human metabolism (Jeevitha *et al.*, 2013). However, vitamin K_1 observed post-monsoon maxima in the ribbon fishes along the SW and SE coasts (3.8 and 2.1 µg/100 g, respectively). Vitamin K plays an important role in blood clotting and bone metabolism pertaining to the prevention of osteoporosis and carotid artery elasticity.

Inter-annual and seasonal variability of macro and micro mineral content in Trichiurus lepturus

The macro (Na, K, Ca and P) and micro (Fe, Mn, Zn, Se) mineral concentrations during the three seasons over the four years from SW and SE coasts of India are depicted in Table 4. No significant inter-annual variations in mineral compositions were observed between the ribbon fish samples collected from the SW and SE coasts over four years (2008 - 2011). The sodium contents in *T. lepturus* ranged from 67 – 933 mg/100 g in SW and 21 - 917 mg/100 g in SE coast. The potassium contents in the edible portion of ribbon fishes ranged from 125 – 1500 mg/100 g in SW and 102 - 318 mg/100 g in SE coast. A high Na/K ratio was observed during the post-monsoon season (0.8 & 1.0, respectively). The calcium content in the ribbon fishes ranged from 90 – 281 mg/100 g in SW and 74 - 318 mg/100 g in SE coast, whilst phosphorus content ranged from 95 – 1045 mg/100 g in SW and 1169 - 1672 mg/100 g in SE coast. The mean concentrations of alkaline metals (Na, K, and Ca) and phosphorus were significantly higher during the monsoon season in the SE coast ($p < 0.05$). The higher content of the macro minerals in the ribbon fishes from the SE coast suggested that the samples from this coast could be used as good sources of these minerals. The variations in the concentration of the different mineral components in the fish could have been as a result of the rate in which these components are available in the water body (Yeannes and Almandos, 2003). A significant variation in the Na content between the ribbon fishes collected from the both coasts were recorded ($p < 0.05$). The current study showed a higher concentration of Na in ribbon fish as compared to the previous findings on marine fish (Nurnadia *et al*., 2013). Similar trends were followed in the case of K, where the highest contents of K were observed in the ribbon fishes collected during the post-monsoon season along the SW coast. However, Na/K ratio observed to be < 2.0, which is interesting from the nutritional point of view (Erkan and Ozden, 2007). The significantly higher K content of ribbon fishes in the present study established the superiority over sea bass (459.7 mg/100 g) and sea bream (393.8 mg/100 g). Balanced contents of Na and K are required to maintain the osmotic balance and pH of the body fluid that in turn regulate the muscle and nerve irritability, control glucose absorption and

Table 4. Macro and micro mineral composition of *T. lepturus* collected from the south west and south east coasts of India during 2008-2011 in three different seasons (pre-monsoon, monsoon and post-monsoon).

	Year	Macroelements					Microelements			
		Na	**K**	**Na/K**	**Ca**	**P**	**Fe**	**Mn**	**Zn**	**Se**
SOUTH WEST COST										
Pre-monsoon	2008	78.19±11.18^{a}	132.84±19^{a}	0.59±0.06^{a}	232.02±33.18^{a}	975.26±139.48^{ac}	1.83±0.26^{a}	0.14±0.02^{ac}	3.63±0.52^{a}	0.08±0.01^{ad}
	2009	72.68±10.4^{a}	124±17.73^{a}	0.59±0.06^{a}	207.25±29.64^{ac}	970.29±138.77^{ac}	1.81±0.26^{a}	0.18±0.03^{ac}	2.28±0.33^{ad}	0.1±0.01^{ad}
	2010	67.18±9.61^{a}	115.15±16.47^{a}	0.58±0.06^{a}	182.47±26.1^{ac}	965.32±138.06^{ac}	1.79±0.26^{a}	0.21±0.03^{a}	0.93±0.13^{ad}	0.11±0.02^{ad}
	2011	89.21±12.76^{a}	150.53±21.53^{a}	0.59±0.06^{a}	281.56±40.27^{ad}	985.2±140.9^{ac}	1.86±0.27^{a}	0.08±0.01^{a}	6.33±0.91^{a}	0.05±0.01^{ad}
Monsoon	2008	72.36±10.35^{a}	108.39±15.5^{a}	0.67±0.06^{a}	159.33±22.79^{ac}	1288.21±184.24^{ac}	1.29±0.18^{a}	0.12±0.02^{ac}	0.67±0.1^{bd}	0.05±0.01^{ad}
	2009	102.91±14.72^{a}	141.3±20.21^{a}	0.73±0.07^{ab}	125.01±17.88^{bc}	644.11±92.12^{a}	1.94±0.28^{a}	0.12±0.02^{ac}	1.09±0.16^{ad}	0.03±0.00^{a}
	2010	87.63±12.53^{a}	124.84±17.85^{a}	0.70±0.06^{ab}	142.17±20.33^{ac}	966.16±138.18^{ac}	1.61±0.23^{a}	0.12±0.02^{ac}	0.88±0.13^{ad}	0.04±0.01^{a}
	2011	133.47±19.09^{a}	174.2±24.91^{a}	0.77±0.07^{ab}	90.68±12.97^{bc}	ND	2.59±0.37^{ad}	0.11±0.02^{ac}	1.51±0.22^{ad}	ND
Post-monsoon	2008	933.61±133.52^{c}	1766.19±252.6^{c}	0.53±0.06^{a}	112.61±16.11^{bc}	1405.02±200.94^{ac}	33.28±4.76^{b}	ND	11.43±1.64^{c}	0.11±0.02^{ad}
	2009	561.33±80.28^{b}	947.63±135.53^{d}	0.59±0.06^{a}	118.38±16.93^{b}	1042.08±149.04^{ac}	17.12±2.45^{c}	0.03±0.00^{b}	7.29±1.04^{e}	0.16±0.02^{bd}
	2010	189.05±27.04^{ad}	129.07±18.46^{a}	1.47±0.15^{b}	124.16±17.76^{b}	679.14±97.13^{a}	0.96±0.14^{a}	0.06±0.01^{bc}	3.14±0.45^{ad}	0.22±0.03^{b}
	2011	375.19±53.66^{bd}	538.35±76.99^{b}	0.70±0.06^{ab}	121.27±17.34^{b}	860.61±123.08^{ac}	9.04±1.29^{d}	0.05±0.01^{b}	5.21±0.75^{a}	0.19±0.03^{b}
SOUTH EAST COAST										
Pre-monsoon	2008	41.44±5.93^{ad}	105.64±15.11^{a}	0.39±0.05^{a}	129±18.45^{a}	ND	1.3±0.19^{a}	0.27±0.04^{a}	0.8±0.11^{a}	0.05±0.01^{ac}
	2009	258.02±36.9^{cd}	333.21±47.65^{c}	0.77±0.07^{b}	74.53±10.66^{a*}	1347.76±192.75^{a}	14.65±2.09^{b}	0.05±0.01^{bc}	0.1±0.01^{a}	0.10±0.01^{ac}
	2010	34.02±4.87^{ad}	102.07±14.6^{a}	0.33±0.05^{a}	142.48±20.38^{a}	ND	1.32±0.19^{a}	ND	0.72±0.1^{a}	0.10±0.01^{ac}
	2011	97.09±13.89^{ad}	145.63±20.83^{a}	0.67±0.06^{ab}	318.37±45.53^{c}	1273.39±182.12^{a}	1.3±0.19^{a}	0.12±0.02^{b}	4.88±0.7^{b}	0.02±0.00^{ac}
Monsoon	2008	917.73±131.25^{b}	627.79±89.78^{b}	1.46±0.15^{b}	ND	1487.84±212.79^{a}	0.79±0.11^{a}	ND	0.11±0.02^{a}	ND
	2009	301.28±43.09^{cd}	347.11±49.64^{c}	0.87±0.08^{b}	267.8±38.30^{b}	1672.62±239.22^{a}	1.07±0.15^{a}	ND	0.8±0.11^{a}	ND
	2010	29.42±4.21^{ad}	88.22±12.62^{a}	0.33±0.05^{a}	105.43±15.08^{a}	1185.17±169.5^{a}	1.05±0.15^{a}	0.05±0.01^{bc}	0.52±0.07^{a}	0.31±0.04^{a}
	2011	165.35±23.65^{ad}	217.66±31.13^{ac}	0.76±0.07^{ab}	186.62±26.69^{a}	1428.89±204.36^{a}	1.06±0.15^{a}	0.03±0.00^{bc}	0.66±0.09^{a}	0.15±0.02^{ac}
	2008	168.1±24.04^{ad}	146.84±21^{a}	1.15±0.1.2^{b}	173.25±24.78^{ad}	1188.93±170.04^{a}	0.67±0.10^{a}	0.15±0.02^{b}	0.77±0.11^{a}	0.89±0.13^{b}
	2009	161.81±23.14^{ad}	141.66±20.26^{a}	1.14±0.12^{b}	166.45±23.81^{a}	1179.23±168.65^{a}	0.79±0.11^{a}	0.13±0.02^{b}	0.72±0.10^{a}	0.95±0.14^{b}
Post-	2010	155.53±22.24^{ad}	136.48±19.52^{a}	1.14±0.12^{b}	159.65±22.83^{a}	1169.52±167.26^{a}	0.91±0.13^{a}	0.1±0.01^{b}	0.66±0.09^{a}	1.01±0.14^{b}
monsoon	2011	148.81±21.28^{ad}	195.9±28.02^{ac}	0.76±0.07^{b}	167.95±24.02^{a}	1286±183.92^{a}	0.95±0.14^{a}	0.02±0.0^{bc}	0.59±0.08^{a}	0.14±0.02^{ac}

Data are expressed as mean ± standard deviation (n = 3); Different superscripts (a-d) within a column denote significant differences ($p < 0.05$). Macro minerals (mg/100 g edible portion) are Na, K, Ca and P; micro minerals (mg/100 g edible partion) are Fe, Mn, and Zn. Se has been expressed in µg/100 g edible portion.

enhance normal retention of protein during growth. A balanced Na/K ratio in the ribbon fishes in the present study, especially during the post-monsoon season appeared to be vital since physiological and epidemiological data recommend that a high Na/K ratio intake can be associated with an increased risk of developing high blood pressure and cardiovascular diseases. A higher phosphorus level was observed throughout the seasons along the SE and SW coasts, especially during the pre/post monsoon season along the SE coast. Phosphorus has been generally associated with the phospholipid content and the presence of phosphoprotein. Phosphorus was found to be the most abundant macro mineral in *T. lepturus*, followed by, potassium, calcium and sodium irrespective of the study locations and seasons.

An extensive variation was recorded in the concentration of Fe contents in the muscles of ribbon fish. Most of the micro minerals were found to be higher in the ribbon fish, including those important as enzyme substrate activators (Mn and Zn) and as metalloenyzme (Fe). In fact, the Fe content reported in this study were much higher than those reported by Belinsky *et al*. (1996), who found that Fe contents ranged between 0.2-0.4 mg/l00 g in whitefish (*Coregonus clupeaformis*). In the present study, the Mn content has shown significantly lower amounts throughout the seasons, and were comparable to the previous findings in fresh fish in Saudi Arabia (0.17 mg/100 g wet sample). The content of Mn in the ribbon fishes were found to be lower than the permissible limit set by FAO/WHO (1984), as 5.4 ppm or 0.54 mg/100 g food. Fe was found to be the most abundant micro element followed by Zn in the ribbon fishes throughout the period studied. Similarly, the ribbon fishes were found to contain Zn lower than the limit set by FAO/WHO (1984) (150 mg/100 g). The Se content in the ribbon fishes was significantly higher than cereals (<10 µg/100 g), fruits and vegetables (<10 µg/100 g) (Levander and Burk, 1994). The abundance of Fe, Mn and Zn among the samples collected from the SW coasts was likely be due to the high bioavailability of these elements arising in the fishes by a high metal absorption from the food chain as a consequence of high feeding activity. The Se concentration in the ribbon fishes from the SW coast ranged from 0.03 - 0.19 µg/100 g, and 0.02 -1.01 µg/100 g in those obtained from the SE coast during the post-monsoon season.

Conclusions

The present work has elucidated more on the importance of *T. lepturus* as good sources of protein, amino acids, minerals and vitamins. The results showed that these fishes are a source of high quality protein, with a well balanced composition of essential amino acids, vitamins and minerals. Further, through this bio-monitoring study, this data can be used as baseline for comparisons in future, with regard to fish nutritional quality. The present study also indicated a reasonably good ratio of essential to non-essential amino acids for ribbon fishes, especially from the SE coast, and therefore, it can be concluded that *T. lepturus* is a good source of well balanced proteins with high-biological value. This study provides a comprehensive information on the inter-annual, spatial and seasonal variations in the protein, amino acid, mineral and vitamin composition of *T. lepturus* in order to utilize this fish species for nutritional and fish processing industries.

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References

AOAC, 2005. In: Latimer, G. W., Horwitz, W. (Eds). Official methods of analysis of the Association of Official Analytical Chemists International. 18th edn. AOAC, Gaithersburg, MD, 473.

Astorga-España, M. S., Rodríguez-Rodríguez, E. M. and Díaz-Romero, C. 2007. Comparison of mineral and trace element concentrations in two molluscs from the Strait of Magellan (Chile). Journal of Food Composition and Analysis 20(3-4): 273-279.

Belinsky, D. L., Kuhnlein, H. V., Yeboah, F., Penn, A. F. and Chan, H. M. 1996. Composition of fish consumed by the James Bay Cree. Journal of Food Composition and Analysis 9(2): 148-162.

Boran, G. and Karacam, H. 2011. Seasonal changes in proximate composition of some fish Species from the Black Sea. Turkish Journal of Fisheries and Aquatic Sciences 11: 1-5.

Chakraborty, K., Joseph, D., Selsa, C. J., and Vijayan K. K. 2013. Inter-annual and seasonal dynamics in amino acid, vitamin and mineral composition of *Sardinella longiceps.* Journal of Food and Nutrition Research 1(6): 145-155.

Chakraborty, K., Joseph D. and Selsa, C. J. 2014. Inter-annual and seasonal dynamics in lipidic signatures of *Sardinella longiceps*. Journal of Aquatic Food Product Technology In Press.

Deosthale, Y. G., Mohan, V. S. and Rao, K. V. 1970. Varietal deficiencies in protein lysine and leucine content of gram sorghum. Journal of Agriculture and Food Chemistry 18: 644-646.

Deutz, N. E. P., Reijven, P. L. M., Athanasas, G. and Soeters, P. B. 1992. Post-operative changes in hepatic, intestinal, splenic and muscle fluxes of amino acids and ammonia in pigs. Clinical Science 83: 607-614.

Erkan, N. and Ozden, O. 2007. Proximate composition and mineral contents in aqua cultured sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) analyzed by ICP-MS. Food Chemistry 102 (3): 721-725.

FAO/WHO. 1984. List of maximum levels recommended for contaminants by the Joint FAO/ WHO Codex Alimentarius Commission Second Series. CAC/FAL, Rome 3: 1-8.

FAO/WHO/UNU. 1985. Energy and protein requirements Report of a joint FAO/WHO/ UNU Expert Consultation, World Health Organization technical report series 724. Geneva: WHO.121-123.

FAO/WHO. 1991. Protein quality evaluation. Report of the joint FAO/WHO Expert Consultation, FAO Food and Nutrition Paper 51, Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO/WHO/UNU. 2007. WHO Technical Report Series 935. Protein and amino acid requirements in human nutrition. Report of a Joint FAO/WHO/UNU Expert Consultation.

Friedman, W. E. 1996. Introduction to biology and evolution of the Gnetales. International Journal of Plant Sciences 157: S1-S2.

García, G. B. and Valverde, C. J. 2006. Optimal proportions of crabs and fish in diet for common octopus (*Octopus vulgaris*) ongrowing. Aquaculture 253: 502-511.

Gulsun, Ö., Esmeray, K., Serhat, O. and Fatih, O. 2009. Sensory, microbiological and chemical assessment of the freshnessof red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. Food Chemistry 114: 505-510.

Heinrikson, L. and Meredith, S.C. 1984. Amino acid analysis by reverse-phase high-performance liquid chromatography precolumn derivatization with phenylisothiocyanate. Analytical Biochemistry 136: 65-74.

Iqbal, A., Khalil, I. A., Atgeeq, N. and Khan, M. S. 2006. Nutritional quality of important food legumes. Food Chemistry 97: 331-335.

Iwasaki, M. and Harada, R. 1985. Proximate and amino acid composition of the roe and muscle of selected marine species. Journal of Food Sciences 50: 1585-1587.

James, P. S. B. R., Alagarswami, K., Rao N. K. K. V., Muthu, M. S., Rajagopalan, M. S., Alagaraja, K. and Mukundan, C. 1986. Potential Marine Fishery Resources of India. Seminar Potential Marine Fishery Resources Mimeo. pp 20.

Jan, U., Shah, M., Manzoor, T. and Ganie, S. A. 2012. Variations of protein content in the muscle of fish *Schizothorax niger.* American Eurasian Journal of Scientific Research 7(1):1-4.

Jeevitha, M., Athiperumalsami, T. and Kumar, V. 2013. Dietary fibre, mineral, vitamin, amino acid and fatty acid content of seagrasses from Tuticorin Bay, Southeast coast of India. Phytochemistry 90: 135-146.

Larsen, R., Eilertsen, K. and Elvevoll, E. O. 2011. Health benefits of marine foods and ingredients. Biotechnology Advances 29: 508-518.

Levander, O. A. and Burk, R. F. 1994. Selenium. In Ziegler, E. E., and Filer, J. J. (Eds). Present knowledge in nutrition, 7th edition. Washington, AC: ILSI press, 320-328.

Lowry, O. H., Roserrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193: 265-275.

Monalisa, K., Islam, M. Z., Khan, T. A., Abdullah, A. T. M. and Hoque, M. M. 2013. Comparative study on nutrient contents of native and hybrid Koi (*Anabas testudineus*) and Pangas (*Pangasius pangasius*, *Pangasius hypophthalmus*) fish in Bangladesh. International Food Research Journal 20(2): 791-797.

Nurnadia, A. A., Azrina, A., Amin, I., Yunus, M. A. S. and Effendi, M. I. H. 2013. Mineral contents of selected marine fish and shellfish from the west coast of Peninsular Malaysia. International Food Research Journal 20(1): 431-437.

Papes, F., Surpili, J. S., Langone, F., Trigo, J. R. and Arruda, P. 2001. The essential amino acid lysine acts as precursor of glutamate in the mammalian central nervous system. FEBS Letters 488: 34-38.

Robbins, C., Felicetti, L. and Fllorin, S. 2010. The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation. Oecologia 162: 571-579.

Salo-Vaananen, P., Mattila, P., Lehikoinen, K., Salmela-Molsa, E. and Piironen, V. 2000. Simultaneous HPLC analysis of fat-soluble vitamins in selected animal products after small-scale extraction. Journal of Agriculture and Food Chemistry 71: S 535-543.

Thiagarajan, R., Lazarus, S., Sastry, A. Y., Khan, Z. M., Kasim, M. H. and Scariah, K. S. 1992. Stock assessment of the ribbon fish, *Trichiurus lepturus* Linnaeus, from Indian waters. Indian Journal of Fisheries 39(3, 4): 182-194.

Trivedi, D. P., Doll, R. and Khaw, K. T. 2003. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. British Medicinal Journal 326: 469-475.

Wu, G. and Morris, S. M. Jr. 1998. Arginine metabolism: nitric oxide and beyond. Biochemical Journal 336:1-17.

Yeannes, I. M. and Almandos, M. E. 2003. Estimation of fish proximate composition starting from water content. Journal of Food Composition and Analysis 16: 81-92.