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Inter Annual and Seasonal Dynamics in Amino Acid, Vitamin and Mineral Composition of *Sardinella longiceps*

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Abstract *Sardinella longiceps* were studied for the spatial (south west (SW) and southeast (SE) coast of India), annual (2008, 2009, 2010 and 2011) and seasonal (pre-monsoon, monsoon and post-monsoon) variations of protein, amino acids, minerals and vitamins. The chlorophyll-a concentration and sea surface temperature of its habitats were taken into account to understand their effect on the nutrient signatures of oil sardine throughout the study period and locations. Mean protein content attained its maximum during pre-monsoon along both SW and SE coasts, with high proportions of essential amino acids. Essential to non-essential amino acid ratio, total aromatic (TArAA) and total sulfated amino acids (TSAA) recorded monsoon maxima along the study locations. Amino acid scores observed monsoon and post-monsoon maxima along the SW and SE coast, respectively. Minerals were significantly higher during monsoon along the SE coast ($P<0.05$). Significant seasonal variations in vitamin content were observed along the study locations with high vitamin A, D_3 and C on SW coast and higher vitamin E and K in SE coast. The present study demonstrated Sardinella longiceps as a valuable source of the protein, amino acids, minerals and vitamins. A reasonably good ratio of essential to nonessential amino acids for oil sardines was recorded throughout different study period and locations, and therefore it can be concluded that this low-value species is an excellent source of good balanced proteins with high-biological value to be qualified as a preferred health food for human diet.

Keywords: *fish, Sardinella longiceps, amino acids, minerals, vitamins, amino acid score*

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1. Introduction

Marine fishes are known to be a superior quality and relatively cheaper protein source rich in essential amino acids, mineral elements, and vitamins [1]. Fish proteins are of high nutritional quality and are fairly balanced with respect to various essential amino acids. The amino acid composition is one of the most important nutritional qualities of protein and the amino acid score [2] is used to evaluate protein quality world-wide. Marine fish is of high biological value with the best balance of the dietary essential amino acids comparing favorably with egg, milk and meat in the nutritional value of its protein [3]. *Sardinella longiceps* Valenciennes, 1847 (Oil Sardine; Family Clupeidae) located in the Indian Ocean (northern and western parts only, Gulf of Aden, Gulf of Oman, but apparently not Red Sea or the "Gulf", eastward to the southern part of India, on the eastern coast to Andhra; possibly to Andamans) [4] is finding more acceptances recently because of its extraordinary nutritional qualities. It is a pelagic fish, which forms prime constituents of India's pelagic fisheries owing to its wide distribution, food/feed value and industrial use of its body oil [5].

The proper understanding about the biochemical constituents of this *S. longiceps* has become a primary requirement for the nutritionists and dieticians. Another vital area which needed the accurate information on biochemical composition is processing industries of fish and fishery products. A perusal of literature shows that very little information is available on the study regarding nutritional composition of oil sardine *viz.* its amino acid, mineral and vitamin content, though it is a dominant fishery resource off Indian coast for the past three decades. Hence this study is designed to examine the spatial (south west (SW) and south east (SE) coast of India), annually (2008, 2009, 2010 and 2011) and seasonal (pre-monsoon, monsoon and post-monsoon) variations of true protein, amino acids, minerals and fat soluble vitamins of *Sardinella longiceps* collected from SW and SE coast of India by studying its living environment *viz.* chlorophyll concentration and sea surface temperature keeping in mind the implication of such a variation for pharmaceutical products, food additives, and dietary health supplements.

2. Materials and Methods

2.1. Samples

Fresh oil sardines Figure 1 were collected (1 kg each) from fishing harbors of Mangalore, Calicut, Cochin (SW coast) and Chennai, Mandapam, Tuticorin (SE coast) during 2008 – 2011on the 15th day of each month. In order to obtain information on the seasonal variations, monthly data were grouped as pre-monsoon (February to May), monsoon (June to September) and post-monsoon (October to January). The results of the three centers in each coast were pooled and average values were used in the present study. Within each sampling two pools of fish per fishing site, each composed of 15–20 specimens of comparable body size were collected, washed in sterile water and brought immediately to the laboratory in an ice box. The whole fish were then gutted and minced for analyses. The time interval between capturing and the arrival of the fish at the landing sites was about 3-4 hours. Although, age and sex differences in nutritional composition evidently could occur, we regarded the fish as a whole food source, which was representative of the market and thus totally used by the local population, without any age or sex differences.

Figure 1. Indicative photograph of *Sardinella longiceps* collected from SW and SE coasts

2.2. Determination of Protein and Amino Acids

The protein contents of the oil sardines were estimated by the established method [6]. 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 protein content of the sample was calculated from the standard curve of bovine serum albumin, and expressed as g/100g wet tissue. The amino acid content of the oil sardines was measured using the Pico - Tag method as described earlier [7] using suitable modifications. 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, 20µL, 7:1: l v/v/v). The derivatized sample (PTC derivative, 20 µL) was diluted with sample diluent (20 µL, 5 mM sodium phosphate $NaHPO_4$ 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 C_{18}, 3.9 X 150 mm) maintained at 38±1°C in a column oven to be detected by their UV absorbance (λ_{max} 254 nm; Waters 2487 dual absorbance detector). The mobile phase eluents 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/100g wet tissue.

2.3. 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 [8] 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 [9].

2.4. Determination of Fat Soluble Vitamins

Estimation of fat soluble vitamins was carried out by a modified method of Salo-Vaananen et al. [10]. The stock solutions of vitamin standards (Sigma-Aldrich Chemical Co. Inc, St. Louis, MO) were prepared (1, 10, 25, 50, & 100 ppm) to construct the standard curve by HPLC. All the stock solutions were stored at -20°C except vitamin D3 where the stock solutions were stored at 4°C. Lipids (0.1 g, t-BHQ 0.02%, w/w) were extracted using established method [11], which was 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 8ml) 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 C_{18} column (Phenomenex, 250 mm length, 4.6 mm I.D., 5µm) in column oven (32°C) and connected to a detector (PDA). The run time was 45 min, and the eluents were detected at 265 nm (UV detector) 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 [12]. The vitamins A, D_3, E, K_1 and C were expressed as µg/100g fresh sample.

2.5. 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 [13]. In brief, samples (2 g) were placed in digestion tubes, to which concentrated HNO_3 (7 ml) was added, and the content was kept for overnight digestion in a fume hood until no brown fumes appeared. The digestion was continued over the sand bath with $HClO_4$ (6 ml) until the color of the solution became pale yellow to colorless. The solution was thereafter cooled and filtered through Whatman No. 1 filter paper. The filtrate was diluted with distilled water (50 ml) to be injected in atomic absorption spectrophotometer for the determination of minerals. The analyses of Ca, Na, K, Mn, Fe, and Zn were performed by flame atomic absorption spectrophotometry equipped with a hollow cathode lamp containing D_2 lamp background correction system. For Se, 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 [12].

2.6. Chlorophyll-a Concentration and Sea Surface Temperature

The chlorophyll-a concentrations were derived from global 9-km monthly mean SeaWiFS (Sea Viewing Wide Field-of-view Sensor) data for the period from January 2008 to December 2011 (*http://reason.gsfc.nasa.gov/OPS/Giovanni/ocean.seawifs.shtml*) to indicate the distribution of the photosynthetic pigment chlorophyll 'a', and expressed as mg/m^3 Figure 2 A-F. Sea surface temperature (SST) was derived from global 9 km monthly mean MODIS (Moderate Resolution Imaging Spectroradiometer) – AQUA data for the period from January 2008 to December 2011 (*http://reason.gsfc.nasa.gov/OPS/Giovanni/ocean.seawifs.shtml*) which represented the temperature at the top 0.1 mm of water column.

2.7. Statistical Analyses

Statistical evaluation was carried out with the Statistical Program for Social Sciences 13.0 (SPSS Inc, Chicago, USA, ver. 13.0). Descriptive statistics were calculated for all the studied traits. 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 biochemical compositions of samples collected was analyzed. The level of significance for all analyses was $p \leq 0.05$. Principal component analysis (PCA) was carried out.

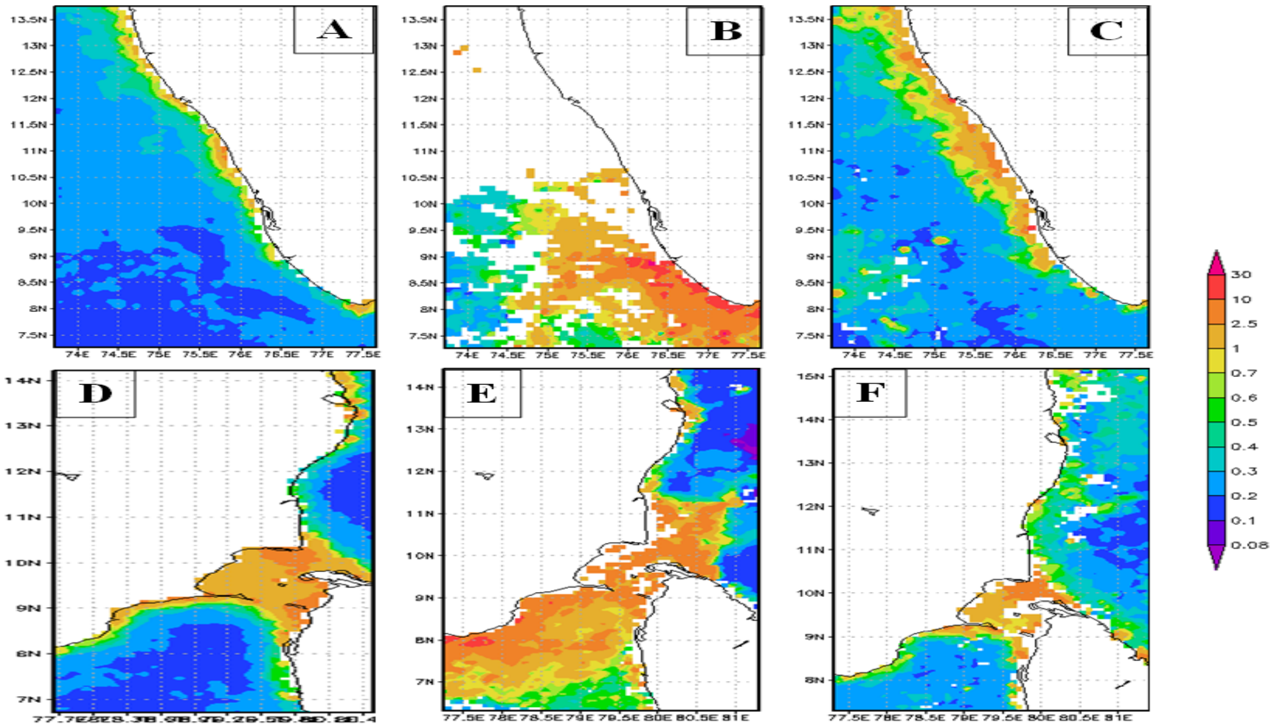

Figure 2. Indicative satellite images of SeaWiFS chlorophyll-a concentration in the year 2009 along Southwest coast of India during (**A**) pre-monsoon, (**B**) monsoon, (**C**) post–monsoon seasons; and Southeast coast of India during (**D**) pre-monsoon, (**E**) monsoon, and (**F**) post–monsoon season.

3. Results

3.1. Inter Annual and Seasonal Variability of Total Protein and Amino acids Content in *S. longiceps* Collected from the South West and South East Coast of India

The true protein content in oil sardines collected from SW and SE coasts is shown in Table 1A and 1B, respectively. The total protein content ranged from 11 - 19.7 g/100g in SW coast samples and between 11 - 15.4 g/100g in SE coast samples. The current study indicated substantial spatial and temporal variation in the true protein content with average total protein content being maximum in pre-monsoon along both SW (19.3 g/100g) and SE (14.4 g/100g) coasts.

The essential, non-essential amino acid compositions of *S. longiceps* from SW and SE coasts are recorded in Table 1 A and 1 B, respectively. Essential amino acids (EAA) dominated the protein content in the sardines from both locations, namely valine, arginine, leucine, lysine etc. No significant differences in the amino acid composition between samples from SW and SE coasts were observed over the studied years (2008 - 2011) ($p > 0.05$). The oil sardine protein contains a broad variety of amino acids and their isomers especially high proportion of the EAA which was about 52 - 56% TAA in SW and 56 – 61% TAA in SE coast. Likewise, NEAA observed about 44 - 48% TAA in SW and 39 - 45% TAA in SE coast. The EAA content was observed maximum during monsoon followed by post-monsoon in SW coast dominating valine, leucine and isoleucine. A similar trend was observed in SE coast with valine, methionine, leucine and lysine as the predominant ones. Concerning non-essential amino acid (NEAA), the most important were glutamic acid, glycine, serine, cysteine in SW coast with maximum NEAA observed in monsoon, followed by post-monsoon and pre-monsoon. On SE coast, glutamic acid, glycine, serine, alanine were found dominant with maximum value observed in monsoon, followed by post-monsoon and pre-monsoon.

Table 1A. Protein (g/100g wet sample) and amino acid composition (g/100g wet sample) of *S. longiceps* collected from south west coast 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
Protein	19.2 ± 0.98^{a}	19.7 ± 1.23^{a}	19.3 ± 1.48^{a}	19.0 ± 1.73^{a}	17.32 ± 1.98^{a}	12.1 ± 0.23^{b}	16.5 ± 2.48^{ab}	14.6 ± 2.73^{ab}	12.4 ± 0.98^{b}	12.4 ± 0.32^{b}	11.25 ± 0.36^{b}	11.01 ± 0.12^{b}
Histidine (His)^{a} (1.9 mg/100g)	0.23 ± 0.01^{a}	0.23 ± 0.03^{a}	0.17 ± 0.07^{a}	0.28 ± 0.08^{a}	0.12 ± 0.12^{a}	0.25 ± 0.25^{a}	0.33 ± 0.01^{a}	0.23 ± 0.02^{a}	0.46 ± 0.01^{a}	0.47 ± 0.01^{a}	0.51 ± 0.001^{a}	0.57 ± 0.02^{a}
Arginine(Arg)^{a}	0.78 ± 0.35^{a}	0.78 ± 0.27^{a}	0.58 ± 0.42^{a}	0.98 ± 0.09^{a}	0.96 ± 0.07^{a}	0.22 ± 0.65^{b}	0.25 ± 0.27^{b}	0.48 ± 0.41^{ab}	0.86 ± 0.08^{a}	0.23 ± 0.009^{b}	0.71 ± 0.038^{a}	0.81 ± 0.02^{a}
Threoninea(Thr) (3.4 mg/100g)	0.31 ± 0.03^{a}	0.31 ± 0.3^{a}	0.25 ± 0.05^{a}	0.37 ± 0.37^{a}	0.16 ± 0.16^{a}	0.13 ± 0.13^{a}	0.21 ± 0.01^{a}	0.17 ± 0.05^{a}	0.39 ± 0.09^{a}	0.34 ± 0.04^{a}	0.32 ± 0.02^{a}	0.38 ± 0.01^{a}
Valinea (Val) (3.5 mg/100g)	0.41 ± 0.01^{a}	0.41 ± 0.01^{a}	0.31 ± 0.01^{a}	0.50 ± 0.5^{a}	1.02 ± 0.03^{b}	1.01 ± 0.01^{b}	1.32 ± 0.01^{b}	1.14 ± 0.014^{b}	0.35 ± 0.05^{a}	0.26 ± 0.06^{a}	0.41 ± 0.01^{a}	0.50 ± 0.02^{a}
Methioninea(Met)	0.23 ± 0.01^{a}	0.23 ± 0.03^{a}	0.18 ± 0.08^{a}	0.28 ± 0.28^{a}	0.09 ± 0.09^{a}	0.07 ± 0.07^{a}	0.09 ± 0.09^{a}	0.08 ± 0.008^{a}	0.29 ± 0.09^{a}	0.43 ± 0.03^{a}	0.19 ± 0.09^{a}	0.50 ± 0.01^{a}
Isoleucinea(Ileu) (2.8 mg/100g)	0.35 ± 0.03^{a}	0.35 ± 0.05^{a}	0.27 ± 0.07^{a}	0.42 ± 0.42^{a}	1.00 ± 0.09^{a}	0.07 ± 0.07^{a}	0.65 ± 0.05^{a}	0.27 ± 0.001^{a}	0.41 ± 0.01^{a}	0.38 ± 0.08^{a}	0.39 ± 0.09^{a}	0.38 ± 0.08^{a}
Leucinea(Leu) (6.6 mg/100g)	0.56 ± 0.01^{ab}	0.56 ± 0.06^{ab}	0.26 ± 0.46^{ab}	0.22 ± 0.67^{ab}	0.36 ± 0.36^{a}	0.24 ± 0.24^{a}	0.23 ± 0.03^{a}	0.28 ± 0.28^{a}	0.65 ± 0.05^{ab}	0.32 ± 0.32^{a}	0.57 ± 0.02^{ab}	0.50 ± 0.01^{ab}
Phenylalaninea(Phe)	0.34 ± 0.01^{a}	0.34 ± 0.04^{a}	0.26 ± 0.26^{a}	0.41 ± 0.01^{a}	1.05 ± 0.01^{b}	1.25 ± 0.01^{b}	1.30 ± 0.03^{b}	1.20 ± 0.05^{b}	0.69 ± 0.04^{a}	0.69 ± 0.06^{a}	0.68 ± 0.02^{a}	0.60 ± 0.01^{a}
Lysinea(Lys) (5.8 mg/100g)	0.69 ± 0.01^{a}	0.69 ± 0.01^{a}	0.51 ± 0.01^{a}	0.86 ± 2.01^{a}	0.68 ± 0.01^{a}	0.61 ± 2.01^{a}	0.62 ± 0.01^{a}	0.75 ± 0.001^{a}	0.56 ± 0.01^{a}	0.22 ± 0.01^{a}	0.75 ± 0.01^{a}	0.50 ± 0.01^{a}
Alanine(Ala)b	0.65 ± 0.01^{a}	0.65 ± 0.02^{a}	0.41 ± 0.01^{a}	0.89 ± 0.01^{a}	0.25 ± 0.01^{a}	0.15 ± 0.01^{a}	0.21 ± 0.02^{a}	0.20 ± 0.01^{a}	0.23 ± 0.01^{a}	0.20 ± 0.02^{a}	0.5 ± 0.01^{a}	0.57 ± 0.01^{a}
Cysteine(Cys) b	0.02 ± 0.03^{a}	0.02 ± 0.02^{a}	0.03 ± 0.03^{a}	0.01 ± 0.00^{a}	0.94 ± 0.04^{b}	0.96 ± 0.06^{b}	0.95 ± 0.05^{b}	0.95 ± 0.95^{b}	0.23 ± 0.03^{a}	0.14 ± 0.14^{a}	0.02 ± 0.02^{a}	0.12 ± 0.00^{a}
Glutamic acid (Glu)b	1.07 ± 0.3^{a}	1.07 ± 0.25^{a}	0.94 ± 0.37^{a}	1.20 ± 0.16^{a}	0.95 ± 0.03^{a}	1.02 ± 0.01^{a}	1.24 ± 0.05^{a}	1.07 ± 0.39^{a}	0.94 ± 0.04^{a}	0.86 ± 0.32^{a}	1.15 ± 0.38^{a}	1.24 ± 0.03^{a}
Glycine(Gly)b	0.60 ± 0.02^{a}	0.66 ± 0.006^{a}	0.52 ± 0.002^{a}	0.68 ± 0.008^{a}	0.06 ± 0.006^{b}	0.07 ± 0.07^{b}	0.09 ± 0.09^{b}	0.07 ± 0.07^{b}	0.68 ± 0.009^{a}	0.16 ± 0.01^{a}	0.33 ± 0.003^{a}	0.56 ± 0.06^{a}
Proline(Pro)b	0.46 ± 0.05^{a}	0.46 ± 0.46^{a}	0.37 ± 0.37^{a}	0.55 ± 0.55^{a}	0.65 ± 0.005^{a}	0.32 ± 0.02^{a}	0.21 ± 0.21^{a}	0.39 ± 0.39^{a}	0.79 ± 0.01^{a}	0.55 ± 0.05^{a}	0.24 ± 0.24^{a}	0.26 ± 0.06^{a}
Serine(Ser)^{b}	0.40 ± 0.02^{a}	0.44 ± 0.04^{a}	0.38 ± 0.08^{a}	0.42 ± 0.02^{a}	0.35 ± 0.05^{a}	0.78 ± 0.05^{a}	0.88 ± 0.008^{a}	0.43 ± 0.003^{a}	0.96 ± 0.01^{a}	0.82 ± 0.02^{a}	0.34 ± 0.004^{a}	0.61 ± 0.01^{a}
Tyrosine(Tyr)^{b}	0.15 ± 0.03^{a}	0.15 ± 0.18^{a}	0.13 ± 0.08^{a}	0.16 ± 0.09^{a}	0.34 ± 0.07^{a}	0.36 ± 0.09^{a}	0.42 ± 0.08^{a}	0.37 ± 0.029^{a}	0.53 ± 0.43^{a}	0.56 ± 0.09^{a}	0.54 ± 0.5^{a}	0.42 ± 0.08^{a}
TEAA	3.90 ± 0.02^{a}	3.90 ± 0.6^{a}	2.79 ± 0.02^{a}	4.32 ± 0.68^{a}	5.44 ± 0.06^{a}	3.85 ± 0.07^{a}	5.00 ± 0.09^{a}	4.60 ± 0.007^{a}	4.66 ± 0.09^{a}	3.34 ± 0.06^{a}	4.53 ± 0.04^{a}	4.74 ± 0.06^{a}
TNEAA	3.35 ± 0.02^{a}	3.45 ± 0.06^{a}	2.78 ± 0.02^{a}	3.91 ± 0.68^{a}	3.54 ± 0.06^{b}	3.66 ± 0.07^{a}	4.00 ± 0.09^{a}	3.48 ± 0.007^{a}	4.36 ± 0.09^{c}	3.29 ± 0.06^{a}	3.12 ± 0.03^{a}	3.78 ± 0.06^{a}
TAA	7.25 ± 0.02^{a}	7.35 ± 0.6^{a}	5.57 ± 0.02^{a}	8.23 ± 0.68^{a}	8.98 ± 0.01^{a}	7.51 ± 0.07^{a}	9.00 ± 0.009^{a}	8.08 ± 0.07^{ba}	9.02 ± 0.09^{a}	6.63 ± 0.06^{ba}	7.65 ± 0.03^{a}	8.52 ± 0.06^{a}
TEAA/TAA	0.54 ± 0.32^{a}	0.53 ± 0.4^{a}	0.50 ± 0.08^{a}	0.52 ± 0.42^{a}	0.61 ± 0.005^{a}	0.51 ± 0.05^{a}	0.56 ± 0.08^{a}	0.57 ± 0.43^{a}	0.52 ± 0.96^{a}	0.50 ± 0.02^{a}	0.59 ± 0.34^{a}	0.56 ± 0.01^{a}
TNEAA/TAA	0.46 ± 0.23^{a}	0.47 ± 0.08^{a}	0.50 ± 0.08^{a}	0.48 ± 0.09^{a}	0.39 ± 0.01^{a}	0.49 ± 0.09^{a}	0.44 ± 0.08^{a}	0.43 ± 0.29^{a}	0.48 ± 0.43^{a}	0.50 ± 0.09^{a}	0.41 ± 0.05^{a}	0.44 ± 0.04^{a}
TEAA/TNEAA	1.16 ± 0.02^{a}	1.13 ± 0.06^{a}	1.00 ± 0.02^{a}	1.10 ± 0.08^{a}	1.54 ± 0.06^{b}	1.05 ± 0.07^{a}	1.25 ± 0.09^{a}	1.32 ± 0.07^{a}	1.07 ± 1.09^{a}	1.02 ± 0.16^{a}	1.45 ± 0.001^{a}	1.25 ± 0.56^{a}
TArAA	0.72 ± 0.05^{a}	0.72 ± 0.46^{a}	0.56 ± 0.07^{a}	0.85 ± 0.05^{a}	1.51 ± 0.05^{a}	1.86 ± 0.02^{a}	2.05 ± 0.21^{a}	1.80 ± 0.39^{a}	1.68 ± 0.09^{a}	1.72 ± 0.05^{a}	1.73 ± 0.24^{a}	1.59 ± 0.06^{a}
TSAA	0.25 ± 0.002^{a}	0.25 ± 0.4^{a}	0.21 ± 0.08^{a}	0.29 ± 0.02^{a}	1.03 ± 0.05^{a}	1.03 ± 0.05^{a}	1.04 ± 0.8^{a}	1.03 ± 0.43^{a}	0.52 ± 0.06^{a}	0.57 ± 0.02^{a}	0.21 ± 0.34^{a}	0.62 ± 0.01^{a}
Arg:Lys	1.13 ± 0.302^{a}	1.13 ± 0.4^{a}	1.14 ± 0.08^{a}	1.14 ± 0.02^{a}	1.41 ± 0.05^{a}	0.36 ± 0.05^{a}	0.40 ± 0.02^{a}	0.64 ± 0.43^{a}	1.54 ± 0.06^{a}	1.05 ± 0.02^{a}	0.95 ± 0.34^{a}	1.62 ± 0.01^{a}
Leu: Ileu	1.60 ± 0.03^{a}	1.6 ± 0.08^{a}	0.96 ± 0.08^{a}	0.52 ± 0.09^{a}	0.36 ± 0.01^{a}	3.43 ± 0.09^{a}	0.35 ± 0.08^{a}	1.04 ± 0.29^{a}	1.59 ± 0.03^{a}	0.84 ± 0.09^{a}	1.46 ± 0.5^{a}	1.32 ± 0.04^{a}
Cys: TSAA	0.08 ± 0.02^{a}	0.08 ± 0.00^{a}	0.14 ± 0.02^{a}	0.03 ± 0.00^{a}	0.91 ± 0.06^{b}	0.93 ± 0.07^{b}	0.91 ± 0.002^{b}	0.92 ± 0.07^{b}	0.44 ± 0.02^{c}	0.25 ± 0.06^{c}	0.1 ± 0.003^{c}	0.19 ± 0.06^{c}

a Essential amino acids; ^{b}Non-essential amino acids; TEAA- Total amino acids; TNEAA – Total non-essential amino acids ; TAA - Total amino acids ; TArAA - Total aromatic amino acids; TSAA - Total sulphur containing amino acids ;Data are expressed as mean ± standard deviation (n = 3); Different superscripts (a-c) within a row denote significant differences ($p < 0.05$). FAO/WHO reference pattern (1990) for evaluating proteins (mg/ 100g) were indicated in parentheses (FAO/WHO, 1990). Tryptophan was not determined.

Table 1B. Protein (g/100g wet sample) and amino acid composition (g/100g wet sample) of *S. longiceps* collected from south east coast 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
Protein	15.26 ± 0.02^{a}	15.36 ± 0.03^{a}	14.25 ± 0.02^{a}	12.56 ± 0.02^{a}	14.25 ± 0.01^{a}	11.25 ± 0.36^{a}	12.25 ± 0.48^{a}	14.21 ± 0.73^{a}	10.25 ± 0.98^{a}	11.02 ± 1.23^{a}	10.25 ± 1.48^{a}	10.02 ± 2.73^{a}
Histidine (His)a (1.9 mg/100g)	0.55 ± 0.01^{a}	1.01 ± 0.04^{a}	0.19 ± 0.002^{b}	0.22 ± 0.00^{b}	0.56 ± 0.00^{ab}	0.29 ± 0.00^{b}	0.35 ± 0.00^{ab}	0.44 ± 0.01^{ab}	0.45 ± 0.00^{ab}	0.46 ± 0.00^{ab}	0.48 ± 0.01^{ab}	0.51 ± 0.00^{ab}
Argininea	0.52 ± 0.06^{a}	0.56 ± 0.05^{a}	0.63 ± 0.07^{a}	0.67 ± 0.01^{a}	0.69 ± 0.08^{a}	0.37 ± 0.09^{a}	0.96 ± 0.08^{a}	0.79 ± 0.02^{a}	0.79 ± 0.06^{a}	0.79 ± 0.02^{a}	0.78 ± 0.02^{a}	0.84 ± 0.02^{a}
Threoninea (3.4 mg/100g)	0.23 ± 0.23^{a}	0.20 ± 0.19^{a}	0.30 ± 0.01^{a}	0.33 ± 0.00^{a}	0.53 ± 0.03^{a}	0.29 ± 0.08^{a}	0.30 ± 0.09^{a}	0.38 ± 0.07^{a}	0.40 ± 0.09^{a}	0.40 ± 0.39^{a}	0.39 ± 0.01^{a}	0.44 ± 0.04^{a}
Valinea(3.5 mg/100g)	0.36 ± 0.35^{a}	0.26 ± 0.25^{a}	0.37 ± 0.36^{a}	0.42 ± 0.42^{a}	0.99 ± 0.01^{a}	1.10 ± 0.01^{b}	0.54 ± 0.04^{ab}	0.54 ± 0.01^{ab}	0.46 ± 0.05^{ab}	0.48 ± 0.07^{ab}	0.49 ± 0.01^{ab}	0.55 ± 0.05^{ab}
Methioninea	0.48 ± 0.00^{a}	0.23 ± 0.00^{a}	0.22 ± 0.00^{a}	0.19 ± 0.003^{a}	0.56 ± 0.01^{a}	0.39 ± 0.01^{a}	0.55 ± 0.02^{a}	0.51 ± 0.005^{a}	0.21 ± 0.01^{a}	0.21 ± 0.01^{a}	0.22 ± 0.00^{a}	0.20 ± 0.00^{a}
Isoleucinea(2.8 mg/100g)	0.52 ± 0.00^{a}	0.26 ± 0.00^{a}	0.32 ± 0.00^{a}	0.40 ± 0.002^{a}	0.78 ± 0.00^{a}	0.35 ± 0.00^{a}	0.32 ± 0.08^{a}	0.30 ± 0.001^{a}	0.49 ± 0.001^{a}	0.47 ± 0.01^{a}	0.45 ± 0.00^{a}	0.50 ± 0.00^{a}
Leucinea(6.6 mg/100g)	0.56 ± 0.06^{ab}	0.29 ± 0.01^{a}	0.53 ± 0.00^{ab}	0.58 ± 0.01^{ab}	1.32 ± 0.02^{b}	0.98 ± 0.01^{ab}	1.11 ± 0.01^{ab}	0.99 ± 0.02^{ab}	0.78 ± 0.08^{ab}	0.72 ± 0.00^{ab}	0.66 ± 0.06^{ab}	0.77 ± 0.01^{ab}
Phenylalaninea	0.79 ± 0.01^{a}	0.33 ± 0.02^{a}	0.32 ± 0.01^{a}	0.31 ± 0.03^{a}	0.9 ± 0.01^{a}	0.58 ± 0.00^{a}	0.65 ± 0.00^{a}	0.71 ± 0.00^{a}	0.39 ± 0.00^{a}	0.38 ± 0.00^{a}	0.36 ± 0.00^{a}	0.40 ± 0.00^{a}
Lysine^{a}(5.8 mg/100g)	0.45 ± 0.01^{a}	0.58 ± 0.01^{a}	0.59 ± 0.01^{a}	0.76 ± 0.01^{a}	1.02 ± 0.02^{a}	1.21 ± 0.03^{a}	1.14 ± 0.06^{a}	0.92 ± 0.02^{a}	0.91 ± 0.01^{a}	0.89 ± 0.01^{a}	0.87 ± 0.07^{a}	0.90 ± 0.02^{a}
Alanine^{b}	4.46 ± 0.01^{a}	3.72 ± 0.01^{a}	3.47 ± 0.01^{a}	3.88 ± 0.01^{a}	7.35 ± 0.02^{b}	5.56 ± 0.03^{ab}	5.92 ± 0.06^{ab}	5.57 ± 0.02^{ab}	4.88 ± 0.01^{ab}	4.80 ± 0.01^{ab}	4.70 ± 0.07^{ab}	5.11 ± 0.02^{ab}
Cysteine^{b}	0.78 ± 0.06^{a}	0.42 ± 0.01^{a}	0.52 ± 0.02^{a}	0.51 ± 0.01^{a}	0.34 ± 0.01^{a}	0.26 ± 0.02^{a}	0.24 ± 0.01^{a}	0.44 ± 0.01^{a}	0.52 ± 0.51^{a}	0.54 ± 0.01^{a}	0.58 ± 0.02^{a}	0.50 ± 0.01^{a}
Glutamic acid^{b}	0.11 ± 0.01^{a}	0.19 ± 0.00^{a}	0.03 ± 0.09^{b}	0.02 ± 0.07^{b}	0.25 ± 0.05^{a}	0.32 ± 0.07^{a}	0.23 ± 0.01^{a}	0.19 ± 0.08^{a}	0.09 ± 0.01^{ab}	0.06 ± 0.01^{b}	0.02 ± 0.00^{a}	0.11 ± 0.06^{a}
Glycine^{b}	1.21 ± 0.19^{a}	1.11 ± 0.01^{a}	1.11 ± 0.01^{a}	1.16 ± 0.01^{a}	0.9 ± 0.02^{a}	0.88 ± 0.01^{a}	1.21 ± 0.01^{a}	1.55 ± 0.01^{a}	1.25 ± 0.01^{a}	1.27 ± 0.01^{a}	1.29 ± 0.02^{a}	1.3 ± 0.02^{a}
Proline^{b}	0.41 ± 0.01^{a}	0.42 ± 0.02^{a}	0.48 ± 0.07^{a}	0.33 ± 0.03^{a}	0.21 ± 0.01^{a}	0.27 ± 0.06^{a}	0.24 ± 0.04^{a}	0.4 ± 0.02^{a}	0.36 ± 0.01^{a}	0.38 ± 0.07^{a}	0.37 ± 0.00^{a}	0.40 ± 0.01^{a}
Serine^{b}	0.37 ± 0.06^{a}	0.33 ± 0.03^{a}	0.32 ± 0.02^{a}	0.26 ± 0.02^{a}	0.36 ± 0.05^{a}	0.36 ± 0.05^{a}	0.36 ± 0.35^{a}	0.5 ± 0.49^{a}	0.30 ± 0.29^{a}	0.3 ± 0.01^{a}	0.29 ± 0.08^{a}	0.32 ± 0.03^{a}
Tyrosine^{b}	0.31 ± 0.01^{a}	0.36 ± 0.05^{a}	0.37 ± 0.06^{a}	0.31 ± 0.01^{a}	0.57 ± 0.06^{a}	0.78 ± 0.08^{a}	0.64 ± 0.04^{a}	0.67 ± 0.06^{a}	0.37 ± 0.06^{a}	0.38 ± 0.01^{a}	0.40 ± 0.09^{a}	0.44 ± 0.04^{a}
TEAA	4.46 ± 0.005^{a}	3.72 ± 0.01^{a}	3.47 ± 0.01^{a}	3.88 ± 0.01^{a}	7.35 ± 0.02^{a}	5.56 ± 0.03^{a}	5.92 ± 0.06^{a}	5.57 ± 0.02^{a}	4.88 ± 0.01^{a}	4.80 ± 0.01^{a}	4.70 ± 0.07^{a}	5.11 ± 0.02^{ac}
TNEAA	3.43 ± 0.01^{a}	3.11 ± 0.01^{a}	2.97 ± 0.01^{a}	2.75 ± 0.02^{a}	3.00 ± 0.01^{a}	3.95 ± 0.01^{a}	3.97 ± 0.01^{a}	4.90 ± 0.01^{a}	3.06 ± 0.01^{a}	3.10 ± 0.00^{a}	3.13 ± 0.02^{a}	3.28 ± 0.14^{a}
TAA	7.89 ± 0.02^{ab}	6.83 ± 0.07^{a}	6.44 ± 0.03^{a}	6.63 ± 0.01^{a}	10.35 ± 0.06^{b}	9.51 ± 0.04^{b}	9.89 ± 0.02^{b}	10.47 ± 0.01^{b}	7.94 ± 0.37^{ab}	7.90 ± 0.00^{ab}	7.83 ± 0.01^{ab}	8.39 ± 0.03^{ab}
TEAA/TAA	0.57 ± 0.07^{a}	0.54 ± 0.27^{a}	0.54 ± 0.07^{a}	0.59 ± 0.27^{a}	0.71 ± 0.07^{a}	0.58 ± 0.27^{a}	0.60 ± 0.27^{a}	0.53 ± 0.27^{a}	0.61 ± 0.27^{a}	0.61 ± 0.27^{a}	0.6 ± 0.01^{a}	0.61 ± 0.07^{a}
TNEAA/TAA	0.43 ± 0.09^{a}	0.46 ± 0.19^{a}	0.46 ± 0.09^{a}	0.41 ± 0.09^{a}	0.29 ± 0.19^{a}	0.42 ± 0.19^{a}	0.40 ± 0.19^{a}	0.47 ± 0.19^{a}	0.39 ± 0.19^{a}	0.39 ± 0.19^{a}	0.4 ± 0.01^{a}	0.39 ± 0.09^{a}
TEAA/TNEAA	1.3 ± 0.02^{a}	1.20 ± 0.06^{a}	1.17 ± 0.06^{a}	1.41 ± 0.06^{a}	2.45 ± 0.07^{a}	1.41 ± 0.04^{a}	1.49 ± 0.01^{a}	1.14 ± 0.04^{a}	1.59 ± 0.07^{a}	1.55 ± 0.08^{a}	1.5 ± 0.01^{a}	1.56 ± 0.01^{ab}
TArAA	1.58 ± 0.56^{a}	1.62 ± 0.28^{a}	0.65 ± 0.53^{b}	0.69 ± 0.08^{b}	1.83 ± 1.32^{ac}	1.95 ± 0.98^{ac}	2.05 ± 0.11^{ac}	2.30 ± 0.99^{c}	1.01 ± 0.78^{a}	1.01 ± 0.00^{a}	1.02 ± 0.06^{a}	1.12 ± 0.01^{a}
TSAA	0.59 ± 0.05^{a}	0.42 ± 0.01^{a}	0.25 ± 0.01^{a}	0.21 ± 0.01^{a}	0.81 ± 0.02^{a}	0.71 ± 0.03^{a}	0.78 ± 0.06^{a}	0.69 ± 0.02^{a}	0.30 ± 0.01^{a}	0.27 ± 0.01^{a}	0.24 ± 0.07^{a}	0.31 ± 0.02^{a}
Arg:Lys	1.16 ± 0.07^{a}	0.97 ± 0.07^{a}	1.07 ± 0.07^{a}	0.88 ± 0.07^{a}	0.68 ± 0.07^{a}	0.31 ± 0.27^{a}	0.84 ± 0.03^{a}	0.86 ± 0.07^{a}	0.87 ± 0.06^{a}	0.89 ± 0.04^{a}	0.90 ± 0.07^{a}	0.93 ± 0.04^{a}
Leu: Ileu	1.08 ± 0.09^{a}	1.12 ± 0.09^{a}	1.66 ± 0.09^{a}	1.45 ± 0.09^{a}	1.69 ± 0.09^{a}	2.80 ± 0.09^{a}	3.47 ± 0.09^{a}	3.30 ± 0.09^{a}	1.59 ± 0.09^{a}	1.53 ± 0.01^{a}	1.47 ± 0.09^{a}	1.54 ± 0.09^{a}
Cys: TSAA	0.19 ± 0.06^{a}	0.45 ± 0.06^{a}	0.12 ± 0.01^{a}	0.10 ± 0.01^{a}	0.31 ± 0.06^{a}	0.45 ± 0.03^{a}	0.29 ± 0.02^{a}	0.28 ± 0.01^{a}	0.30 ± 0.01^{a}	0.22 ± 0.01^{a}	0.08 ± 0.01^{a}	0.35 ± 0.06^{a}

Data are expressed as mean ± standard deviation (n = 3). All notations are as indicated in Table 1

3.2. Inter Annual and Seasonal Variability of Nutritional Indices in *S. longiceps* Collected from the South West and South East Coast of India

The nutritional indices with respect to different amino acid ratios of *S. longiceps* collected from SW and SE coast of India is shown in Table 1A and 1B, respectively. EAA/NEAA ratio observed maximum during monsoon in both SW (1.3) and SE (1.6) coasts of India. The EAA/TAA ratio observed monsoon maxima (0.56) in SW coast and monsoon/post-monsoon maxima in SE coast (0.61). Apparently, high NEAA/TAA ratio was observed maximum during pre-monsoon ($\geq$ 0.44) along both coasts. The average total aromatic amino acids (TArAA) showed monsoon maxima in both the coasts (1.8 g/100g in SW & 2.0 g/100g in SE). The average total sulfated amino acids (TSAA = cysteine + methionine) showed higher values in monsoon (1.0 & 0.8 g/100g in SW & SE, respectively). Cys: TSAA ratio also showed higher values in monsoon along both coasts (0.8 & 0.3 in SW & SE, respectively). Correspondingly, in both the coasts, average leucine: isoleucine ratio showed higher values in monsoon/post-monsoon (1.3 g/100g) and monsoon in SE coast (2.8 g/100g). Arg: Lys ratio observed high values in pre-monsoon and post-monsoon in both the coasts. The amino acid scores (with respect to His, Thr, Val, Met+Lys, Ileu, Leu, Phe +Tyr and Lys) of *S. longiceps* collected from SW and SE coast of India is shown in Table 2. The amino acid scores were observed to be higher during monsoon with respect to TSAA, valine, isoleucine, phe+tyr along SW coast. However during post-monsoon, amino acid scores with respect to histidine, threonine, valine, isoleucine, leucine and lysine observed maximum along the SE coast.

Table 2. Essential amino acid scores (%) of *S. longiceps* collected from south west coast and south east coast of India during 2008-2011 in three different seasons.

Amino acids	Pre-monsoon				Monsoon				Post-monsoon			
	2008	2009	2010	2011	2008	2009	2010	2011	2008	2009	2010	2011
SOUTH WEST COAST												
His (1.9)	63	61	46	78	36	109	105	83	195	199	239	272
Thr (3.4)	47	46	38	57	27	32	37	34	93	81	84	102
Val (3.5)	61	59	46	75	168	238	229	223	81	60	104	130
Met + Cys (2.5)	52	51	44	61	238	341	252	282	168	184	75	225
Ile (2.8)	65	63	50	79	206	21	141	66	118	109	124	123
Leu (6.6)	44	43	20	18	31	30	21	29	79	39	77	69
Phe + Tyr (6.3)	41	39	32	48	127	211	165	171	156	160	172	147
Lys (5.8)	62	60	46	78	68	87	65	89	78	31	115	78
SOUTH EAST COAST												
His (1.9)	190	346	70	92	207	136	150	163	231	220	246	268
Thr (3.4)	44	38	62	77	109	76	72	79	115	107	112	129
Val (3.5)	67	48	74	96	199	279	126	109	128	124	137	157
Met + Cys (2.5)	155	109	70	67	227	252	255	194	117	98	94	124
Ile (2.8)	122	60	80	114	195	111	93	75	171	152	157	178
Leu (6.6)	56	29	56	70	140	132	137	106	115	99	98	116
Phe + Tyr (6.3)	107	63	51	59	141	234	220	208	87	79	84	97
Lys (5.8)	51	65	71	104	123	185	160	112	153	139	146	155

The reference value for adults standard FAO/WHO (1991) (g/100 g protein) are given in parentheses.

3.3. Inter Annual and Seasonal Variability of Vitamin Content in *S. longiceps* Collected from the South West and South East Coast of India

The vitamin content of *S. longiceps* collected from SW and SE coast of India is shown in Table 3. No significant differences ($p > 0.05$) in vitamin A, D, E and K content were observed between the samples collected from both SW and SE coast over four years (2008 - 2011). The mean vitamin A content was significantly higher during post-monsoon for the samples collected from both SW (> 8.0 µg/100g) and SE (~ 8.0 µg/100g) coast compared with pre-monsoon and monsoon (< 5.0 µg/100g). The vitamin D content in samples collected from SW coast was significantly higher in monsoon (~ 868 µg/100g) while for SE samples pre-monsoon maxima (~ 391 µg/100g) was observed. A pre-monsoon maxima in SW coast (1.3 µg/100g) and monsoon maxima in SE coast (0.93 µg/100g) were observed for vitamin E content. However, vitamin K observed post-monsoon maxima along both SW and SE coasts (~ 3.5 µg/100g). Apparently, vitamin C content was observed to be high during monsoon in SW and pre-monsoon in SE coasts (12.6 & 11.2 µg/100g, respectively). In general except, vitamin E and K, all other vitamins were observed to be higher in SW coast sardines.

Table 3. Vitamin compositions of *S. longiceps* collected from south west and south east coast of India during 2008-2011 in three different seasons

		Vit A	Vit D_3	Vit E	Vit K	Vit C
		SOUTH WEST COAST				
Pre-monsoon	2008	4.1 ± 0.11^{a}	458.0 ± 0.22^{a}	1.3 ± 0.52^{a}	0.2 ± 0.005^{a}	11.4 ± 0.52^{a}
	2009	3.9 ± 0.10^{a}	452.0 ± 1.12^{a}	1.3 ± 0.62^{a}	0.2 ± 0.001^{a}	11.4 ± 1.13^{a}
	2010	4.2 ± 0.12^{a}	458.0 ± 1.25^{a}	1.3 ± 0.12^{a}	0.2 ± 0.003^{a}	11.2 ± 0.22^{a}
	2011	4.2 ± 0.02^{a}	465.0 ± 0.26^{a}	1.3 ± 0.02^{a}	0.2 ± 0.004^{a}	11.6 ± 0.39^{a}
Monsoon	2008	5.2 ± 0.06^{ac}	865.0 ± 0.22^{b}	0.5 ± 0.16^{b}	0.9 ± 0.001^{b}	12.7 ± 0.38^{a}
	2009	4.3 ± 0.04^{ac}	864.0 ± 0.36^{b}	0.9 ± 0.17^{ab}	1.0 ± 0.01^{b}	12.5 ± 0.35^{a}
	2010	5.0 ± 0.06^{ac}	862.0 ± 0.25^{b}	0.9 ± 0.09^{ab}	1.0 ± 0.05^{b}	12.6 ± 0.23^{a}
	2011	5.3 ± 0.05^{ac}	878.0 ± 0.11^{b}	1.0 ± 0.02^{a}	1.4 ± 0.06^{b}	12.5 ± 0.32^{a}
Post-monsoon	2008	8.3 ± 0.12^{bc}	325.0 ± 0.12^{a}	0.9 ± 0.12^{a}	3.6 ± 0.02^{c}	6.5 ± 0.36^{b}
	2009	8.2 ± 0.11^{bc}	312.0 ± 0.16^{a}	0.9 ± 0.05^{a}	3.2 ± 0.16^{c}	6.2 ± 0.06^{b}
	2010	8.2 ± 0.09^{bc}	212.0 ± 0.02^{c}	0.1 ± 0.001^{c}	3.6 ± 0.13^{c}	5.5 ± 0.22^{b}
	2011	8.2 ± 0.01^{bc}	283.0 ± 1.06^{a}	0.7 ± 0.002^{ab}	3.5 ± 0.12^{c}	6.1 ± 0.22^{b}
		SOUTH EAST COAST				
Pre-monsoon	2008	3.0 ± 0.06^{a}	425.6 ± 1.14^{a}	0.9 ± 0.0^{a}	0.6 ± 0^{a}	11.4 ± 0.11^{a}
	2009	2.6 ± 0.05^{a}	418.8 ± 0.3^{a}	0.9 ± 0.01^{a}	0.4 ± 0.01^{a}	13.8 ± 0.17^{a}
	2010	2.3 ± 0.12^{a}	412.0 ± 0.22^{a}	0.8 ± 0.001^{a}	0.2 ± 0.03^{a}	16.2 ± 0.18^{a}
	2011	3.3 ± 0.04^{a}	306.0 ± 0.18^{a}	0.1 ± 0^{b}	0.6 ± 0.04^{a}	3.5 ± 0.12^{b}
Monsoon	2008	4.0 ± 0.06^{a}	125.0 ± 0.12^{b}	1.0 ± 0.0.03^{a}	1.8 ± 0.002^{b}	9.6 ± 0.18^{a}
	2009	4.0 ± 0.09^{a}	306.0 ± 0.13^{ac}	0.9 ± 0.01^{a}	1.5 ± 0.002^{b}	3.5 ± 0.05^{b}
	2010	4.9 ± 0.07^{a}	265.2 ± 0.04^{c}	0.9 ± 0^{a}	1.2 ± 0.06^{b}	3.3 ± 0.14^{b}
	2011	4.1 ± 0.04^{a}	250.1 ± 0.04^{c}	0.9 ± 0^{a}	1.2 ± 0.04^{b}	3.4 ± 0.02^{b}
Post-monsoon	2008	8.2 ± 0.04^{b}	320.6 ± 0.12^{ac}	0.9 ± 0.01^{a}	3.5 ± 0.002^{c}	6.3 ± 0.04^{ab}
	2009	8.4 ± 0.06^{b}	325.7 ± 0.12^{ac}	0.9 ± 0.02^{a}	3.2 ± 0.002^{c}	6.6 ± 0.02^{ab}
	2010	7.2 ± 0.03^{b}	312.0 ± 0.12^{ac}	0.9 ± 0.003^{a}	3.9 ± 0.002^{c}	6.2 ± 0.02^{ab}
	2011	7.5 ± 0.01^{b}	321.4 ± 0.12^{ac}	0.9 ± 0^{a}	3.6 ± 0.03^{c}	6.1 ± 0.02^{ab}

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

Table 4. Macro and micro mineral composition of *S. longiceps* collected from south west and south east coast of India during 2008-'11 in three different seasons

Season	Year	Na	K	Na/K	Ca	P	Ca /P	Ca+P	Fe	Mn	Zn	∑micro	Se (µg/100 g)
SOUTHWEST COAST													
Pre-monsoon	2008	72.80 ± 0.83^{a}	158.9 ± 1.13^{a}	0.46 ± 0.02^{a}	229.5 ± 0.23^{a}	459.6 ± 1.13^{a}	0.5	689.2 ± 0.25^{a}	270 ± 11.3^{a}	20.0 ± 0.13^{a}	4450 ± 223^{ab}	4740 ± 113^{a}	10 ± 0.25^{a}
	2009	68.20 ± 1.03^{a}	156.2 ± 0.16^{a}	0.44 ± 0.01^{a}	200.1 ± 1.13^{a}	450.3 ± 0.13^{a}	0.4	650.4 ± 1.52^{a}	260 ± 15.2^{a}	20.0 ± 0.11^{a}	3430 ± 116^{a}	3710 ± 125^{a}	10 ± 0.25^{a}
	2010	62.60 ± 0.03^{a}	125.3 ± 0.14^{a}	0.50 ± 0.02^{a}	143.5 ± 0.45^{b}	458.6 ± 2.13^{a}	0.3	602.1 ± 1.25^{a}	260 ± 13.4^{a}	20.0 ± 0.03^{a}	3230 ± 115^{a}	3510 ± 266^{a}	10 ± 0.11^{a}
	2011	87.70 ± 01.13^{b}	195.2 ± 2.15^{b}	0.45 ± 0.09^{a}	344.9 ± 0.55^{c}	431.2 ± 1.23^{a}	0.8	776.1 ± 2.26^{a}	300 ± 12.5^{a}	20.0 ± 0.04^{a}	3700 ± 214^{a}	4020 ± 263^{a}	10 ± 0.03^{a}
Monsoon	2008	112.0 ± 0.15^{c}	310.9 ± 2.13^{c}	0.36 ± 0.09^{a}	374.3 ± 0.22^{c}	293.6 ± 0.88^{b}	1.3	667.9 ± 1.24^{a}	620 ± 33.2^{b}	100 ± 2.13^{b}	5630 ± 214^{b}	6350 ± 113^{b}	10 ± 0.05^{a}
	2009	115.6 ± 0.15^{c}	277.5 ± 1.13^{c}	0.42 ± 0.02^{a}	369.3 ± 0.63^{c}	295.3 ± 1.25^{b}	1.3	664.6 ± 1.33^{a}	650 ± 23.5^{b}	100 ± 1.13^{b}	6130 ± 116^{bc}	6880 ± 118^{b}	10 ± 0.02^{a}
	2010	112.6 ± 0.15^{c}	265.5 ± 2.13^{c}	0.42 ± 0.05^{a}	369.3 ± 0.74^{c}	292.5 ± 1.24^{b}	1.3	661.8 ± 2.14^{a}	650 ± 12.5^{b}	80.0 ± 0.15^{b}	5750 ± 113^{bc}	6480 ± 124^{b}	10 ± 0.23^{a}
	2011	120.6 ± 2.15^{c}	389.6 ± 2.23^{d}	0.31 ± 0.04^{a}	194.3 ± 0.88^{a}	293.0 ± 1.35^{b}	0.7	487.3 ± 2.22^{b}	600 ± 69.0^{b}	100 ± 1.13^{b}	5620 ± 118^{bc}	6320 ± 113^{b}	10 ± 0.01^{a}
Post-monsoon	2008	90.56 ± 0.16^{abc}	214.3 ± 4.13^{bc}	0.42 ± 0.06^{a}	156.8 ± 1.23^{b}	152.8 ± 1.44^{c}	1.0	309.6 ± 0.56^{c}	1000 ± 0.13^{c}	170 ± 0.13^{c}	2440 ± 119^{d}	3610 ± 124^{a}	10 ± 0.02^{a}
	2009	96.65 ± 0.13^{bc}	234.6 ± 1.13^{bc}	0.41 ± 0.04^{a}	148.3 ± 1.12^{b}	164.2 ± 1.42^{c}	0.9	312.5 ± 1.26^{c}	890 ± 15.°C	150 ± 0.13^{c}	2220 ± 118^{d}	3260 ± 156^{a}	10 ± 0.03^{a}
	2010	97.89 ± 0.94b^{c}	122.9 ± 3.53^{a}	0.80 ± 0.08^{a}	186.7 ± 1.05^{a}	178.5 ± 1.03^{c}	1.0	365.2 ± 1.24^{bc}	450 ± 29.3^{d}	20.0 ± 0.13^{a}	1990 ± 318^{d}	2460 ± 203^{a}	20 ± 0.05^{b}
	2011	92.52 ± 0.14^{bc}	230.5 ± 1.88^{c}	0.4 ± 0.002^{b}	171.7 ± 2.13^{ab}	177.4 ± 2.15^{c}	1.0	349.1 ± 2.14^{bc}	1000 ± 11.3^{c}	100 ± 0.13^{b}	2320 ± 219^{d}	3420 ± 105^{a}	20 ± 0.06^{b}
SOUTHEAST COAST													
Pre-monsoon	2008	61.6 ± 0.13^{a}	110.2 ± 0.59^{a}	0.56 ± 0.02^{a}	105.3 ± 0.01^{a}	241.2 ± 0.14^{a}	0.4	346.5 ± 1.2^{a}	270 ± 23.0^{a}	30.0 ± 0.15^{a}	3130 ± 115^{a}	3430 ± 325^{a}	20 ± 0.02^{a}
	2009	59.2 ± 0.13^{b}	93.6 ± 0.56^{a}	0.63 ± 0.001^{a}	107.8 ± 0.13^{a}	251.3 ± 0.13^{a}	0.4	358.8 ± 1.3^{a}	170 ± 16.5^{b}	40.0 ± 0.13^{a}	3790 ± 112^{b}	4000 ± 113^{b}	30 ± 0.06^{b}
	2010	58.1 ± 0.14^{a}	70.7 ± 1.25^{b}	0.82 ± 0.08^{ab}	117.7 ± 0.05^{a}	230.2 ± 0.66^{a}	0.5	347.7 ± 2.13^{a}	160 ± 25.4^{b}	40.0 ± 0.16^{a}	4260 ± 114^{c}	4460 ± 214^{b}	10 ± 0.01^{c}
	2011	71.5 ± 0.13^{c}	136.8 ± 0.13^{c}	0.52 ± 0.02^{ab}	120.56 ± 0.24^{a}	256.6 ± 2.54^{a}	0.5	377.2 ± 0.96^{a}	570 ± 11.3^{c}	40.0 ± 0.13^{a}	3810 ± 165^{b}	4420 ± 213^{b}	10 ± 0.03^{c}
Monsoon	2008	125.56 ± 0.13^{b}	460.6 ± 0.13^{d}	0.27 ± 0.02^{a}	725.56 ± 0.22^{b}	495.6 ± 1.25^{b}	1.5	1221 ± 2.13^{b}	130 ± 12.1^{b}	10.0 ± 0.22^{b}	2110 ± 113^{d}	2250 ± 196^{b}	10 ± 0.02^{c}
	2009	126.36 ± 0.25^{b}	463.8 ± 0.54^{d}	0.27 ± 0.02^{a}	705.89 ± 0.13^{b}	488.5 ± 0.13^{b}	1.4	1194 ± 1.24^{b}	200 ± 11.7^{b}	20.0 ± 0.58^{b}	2180 ± 211^{d}	2400 ± 115^{b}	10 ± 0.05^{c}
	2010	136.54 ± 0.13^{b}	445.5 ± 1.25^{d}	0.31 ± 0.05	720.14 ± 0.13^{b}	487.2 ± 2.35^{b}	1.5	1207 ± 1.25^{b}	120 ± 12.3^{b}	10.0 ± 2.13^{b}	2130 ± 325^{d}	2260 ± 125^{b}	10 ± 0.06^{c}
	2011	130.25 ± 0.22^{b}	444.21 ± 0.13^{d}	0.29 ± 0.02^{a}	726.36 ± 0.13^{b}	485.2 ± 0.13^{b}	1.5	1211 ± 2.13^{b}	100 ± 12.5^{b}	30.0 ± 1.13^{a}	1110 ± 123^{e}	1240 ± 325^{c}	10 ± 0.01^{c}
Post-monsoon	2008	100.25 ± 0.13^{c}	110.2 ± 0.13^{a}	0.91 ± 0.04	562.1 ± 0.04^{c}	352.6 ± 2.15^{c}	1.6	914.7 ± 0.13^{c}	260 ± 21.4^{a}	20.0 ± 1.25^{a}	3560 ± 153^{b}	3840 ± 213^{a}	10 ± 0.08^{c}
	2009	105.25 ± 0.16^{c}	115.2 ± 0.32^{a}	0.91 ± 0.02^{ab}	582.4 ± 0.05^{c}	361.2 ± 1.13^{c}	1.6	943.6 ± 1.93^{c}	290 ± 32.5^{ad}	30.0 ± 0.13^{a}	4560 ± 113^{c}	4880 ± 231^{b}	10 ± 0.07^{c}
	2010	105.65 ± 0.03^{c}	115.8 ± 0.3^{a}	0.91 ± 0.01^{b}	556.9 ± 0.08^{c}	324.5 ± 0.13^{c}	1.7	881.4 ± 1.24^{c}	350 ± 12.4^{d}	30.0 ± 0.33^{a}	4860 ± 133^{c}	5240 ± 125^{b}	30 ± 0.09^{b}
	2011	109.65 ± 0.35^{bc}	113.7 ± 0.13^{a}	0.96 ± 0.00^{ab}	567.1 ± 0.09^{c}	333.7 ± 0.13^{c}	1.7	900.8 ± 1.25^{b}	300 ± 5.6^{ad}	30.0 ± 0.22^{a}	4872 ± 122^{c}	5202 ± 213^{b}	20 ± 0.09^{a}

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/100g wet sample) are Na, K, Ca and P; Micro minerals (µg/100g wet sample) are Fe, Mn, and Zn.

3.4. Inter Annual and Seasonal Variability of Macro and Micro Mineral Content in *S. longiceps* Collected from the South West and South East Coast of India

The macro (Na, K, Ca and P) and micro (Fe, Mn, Zn, Se) mineral concentrations during the three seasons over four years from SW and SE coast of India are depicted in Table 4**.** No remarkable variations in mineral compositions were observed between the samples collected from SW and SE coasts over four years (2008 - 2011) during pre-monsoon, monsoon and post-monsoon. A high Na/K ratio was observed during post-monsoon for samples collected from both SW and SE coasts (1.4 & 1.0, respectively). The mean concentrations of alkaline metals (Na, K, Ca) and phosphorus were significantly higher during monsoon in the SE coast. The values of Ca+P ratio obtained in all the samples from SW coast were in the range 310 – 776 mg/100g in SW and 347 – 1221 mg/100g in SE coast. Fe (260 - 10000 µg/100g), Mn (20 -170 µg/100g) and Zn (1990 – 6130 µg/100g) are more abundant in the samples collected from the SW coasts. Se concentration ranged from 10 - 20 µg/100g in SW coast and 10 -30 µg/100g in SE coast and was high during the post-monsoon along both the coasts.

3.5. Interannual and Variability in Chlorophyll-a Concentration and Sea Surface

Temperature (SST) Along South West and South East Coast of India

The variance in the spatial distribution of chlorophyll–a during 2008- 2011 during pre-monsoon, monsoon and post-monsoon seasons have been computed to examine the uniformity in the distribution of chlorophyll–a over SW coast and SE coast of India and recorded in Figure 3A. On SW coast, chlorophyll-a showed relatively low values in pre-monsoon (4-year pre-monsoon average 0.3 ± 0.02 mg/m^3), reached monsoon maxima (1.2 ± 0.34 mg/m^3), subsequently decreased throughout the post-monsoon season (0.5 ± 0.07 mg/m^3). On SE coast, along monsoon and post-monsoon, similar value in chlorophyll-a was observed (~ 0.8 mg/m^3) and exhibited pre-monsoon minima (0.7 ± 0.17 mg/m^3). Though insignificant, some anomalies are found in the chlorophyll-a content over the years; for eg. on SW coast, 1.58 mg/m^3 during 2008 monsoon, 0.76 mg/m^3 during 2009 monsoon, in SE coast, > 0.8 mg/m^3 during 2008, 2009 and 2011 in post-monsoon but 0.6 mg/m^3 in 2010 post-monsoon. In the SW and SE coasts, the area-averaged SST Figure. 3B during pre-monsoon was the highest observed in all the studied years (> 30°C), which decreased in monsoon (29.5 ± 0.61 and 29.4 ± 0.48°C in SW & SE coast, respectively) and showed a gradual decrease by post-monsoon (< 29°C) in both the coasts.

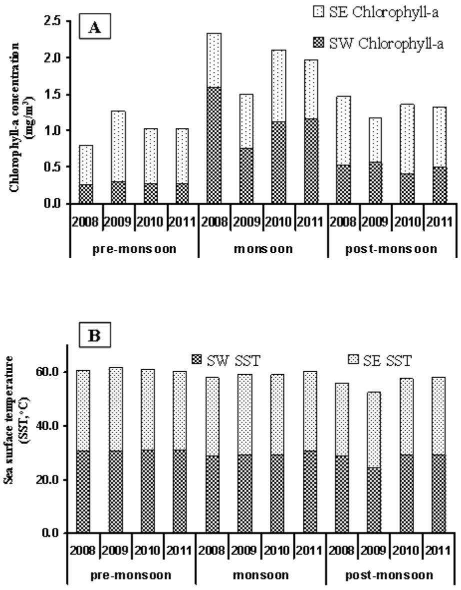

Figure 3. (**A**) Seasonal variability in SeaWiFS chlorophyll-a concentration (mg /m^3) for the study period, 2008 – 2011 (**B**) Variability in sea surface temperature (SST) for the four years (2008 -2011).

3.6. Principal Component Analyses

Principal component analyses (PCA) were performed to determine similarities between the Protein, TEAA, TNEAA, TAA, TArAA, TSAA with chlorophyll-a and SST during pre-monsoon season were shown in Figure 4A. The PC1 explains 42.9% variance which accounts for similarities between the protein content, TNEAA and TArAA in the SE coast. The PC2 axis which explained 36.9% variance gives the highest percentages of TEAA and TSAA along SW coast and PC3 explained 2.15% variance gives the highest percentages of chlorophyll-a along both coasts. PCA were performed to determine similarities between the Protein, TEAA, TNEAA, TAA, TArAA, TSAA with chlorophyll-a and SST during monsoon season were shown in Figure 4B. The PC1 explains 55.6% variance which accounts for similarities between the SST, TNEAA and TArAA in the SE coast and the PC2 axis which explained 22.5% variance gives the highest percentages of protein, chlorophyll-a, EAA, EAA/NEAA along SW coast. PC3 explained 19.9% of the variance which accounts for similarities between the TNEAA, TSAA and TArAA in the SW coast. Similarly, PCA were performed to determine similarities between the Protein, TEAA, TNEAA, TAA, TArAA, TSAA with chlorophyll-a and SST during post-monsoon season were shown in Figure 4C. The PC1 explains 41.0% variance which accounts for similarities between the chlorophyll-a, TNEAA, TSAA in SW coast; EAA/NEAA and TSAA in SE coast and the PC2 (38.1%) related mainly to the SST and TEAA along the SW coast with high positive factor loadings. PC3 explained 21% of the variance which accounts for similarities between the TNEAA, TEAA and TArAA in the SE coast.

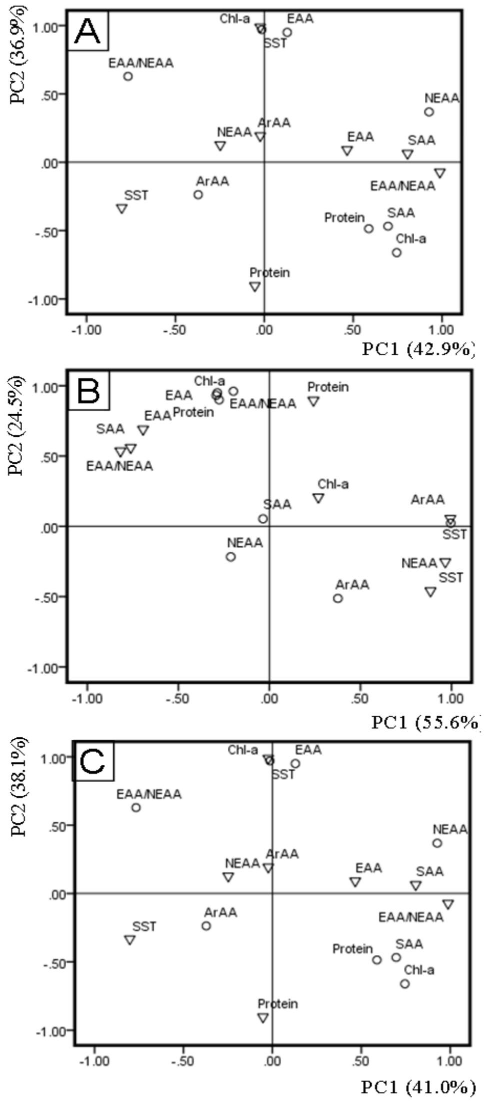

Figure 4. The correlation plots between (**A**) Protein, amino acids., TEAA, TNEAA, EAA/NEAA, TArAA, TSAA, chlorophyll-a and SST during pre-monsoon, (**B**) Protein, amino acids., TEAA, TNEAA, EAA/NEAA, TArAA, TSAA, chlorophyll-a and SST during monsoon, (**C**) Protein, amino acids., TEAA, TNEAA, EAA/NEAA, TArAA, TSAA, chlorophyll-a and SST during post-monsoon.

4. Discussion

Proteins are an essential nutritional component of *S. longiceps*, and are essentially required by the human beings for growth and survival. The high protein content in both coasts was in pre-monsoon and the lowest was in post-monsoon that was in agreement with previously reported results by Chandrashekhar et al. [14] and Jan et al. [3]. This variation in the total protein content can be attributed to the feeding of different protein rich marine phytoplanktons and to climatic changes, which influence the general biochemical composition of the fish [15]. The significantly higher protein content irrespective of the low chlorophyll-a content in pre-monsoon can be due to a higher intake of food than other seasons. In addition, as no gonadal elements are present in pre-monsoon, the food that is consumed is used in the building up of the muscle. During the peak spawning season, i.e., during monsoon in both coasts the protein content was low compared to pre-monsoon because during spawning period the fishes has been found to be active and agile and this results in the utilization of some of the muscle reserves of energy results in decline of muscle protein [3]. Apparently the chlorophyll- a concentration in monsoon showed high correlation with the protein content, especially in SW coast. 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 [3]. In general, the seasonal profile of the protein from oil sardines collected from both the coasts illustrated the active growing phase during monsoon and a decrease after spawning, in post-monsoon.

The amino acid profile of oil sardines from both coasts showed that all the essential amino acids were significantly higher in concentrations, when compared with the reference pattern [2], which implied that the proteins present had a high biological value, and are therefore called complete proteins. The increase of TEAA in monsoon was mainly due to the significant increase of valine and phenyl alanine in this season ($p < 0.05$) along SW coast. Likewise, the high content of TEAA in monsoon compared with pre-monsoon and post-monsoon along the SE coast was due to the considerable increase of valine, leucine and isoleucine in this season ($p < 0.05$). These essential amino acids are called branched chain amino acids (BCAA) which are critical to human life and are particularly involved in stress, energy and muscle metabolism. A high correlation between chlorophyll-a and TEAA (r^2 = > 0.92) during the monsoon clearly showed the outcome of the planktonic diet in the composition of EAAs Figure 4B. On both the coasts, an increase in the serine level, an amino acid in the human body which assists the function of the central nervous system (CNS) was observed during monsoon. Interestingly, oil sardines collected from the SE coast (monsoon and post-monsoon) and SW coast (during pre-monsoon and monsoon) possess high lysine content which is severely restricted in cereals, the most important staple food in the world. In diets based mainly on cereals, a supplement of fish can, therefore, significantly raise the biological value. A reduced supply of lysine in the diet may lead to mental and physical handicaps because it is an important precursor for the *de novo* synthesis of glutamate, the most significant neurotransmitter in the mammalian central nervous system [1].

The percentage ratios of TEAA to TAA (TEAA/TAA) in the samples 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 [6]. The EAA/NEAA ratio which observed more than 1.0 during the monsoon (> 1.2) of SW and monsoon & post-monsoon off SE coast (> 1.6) samples indicated that oil sardine 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 excellent, and therefore it can be concluded that oil sardines from both coasts, especially SE coast are sources of well balanced proteins and high-quality protein source in respect of EAA/NEAA ratio. The EAA/NEAA ratio observed by Iwasaki and Harada [16] was considerably lower for other marine species like *Pagrus major* (0.77), *Scomber japonicus* (0.77), *O. keta* and *Paralichthys olivaceus* (0.77) compared to the present study. The amount of total aromatic amino acids (TArAA) was observed to be high during monsoon in both the coasts. 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. Arg:Lys ratio is the most objective index to identify the cholesterolemic properties of a protein presenting an intermediate ratio between vegetables and meat [17]. The leucine/isoleucine ratios of all the sardines from both the coasts were typical, of the ideal ratio suggested by FAO/ WHO [10]. Deosthale et al. [18] showed that excess leucine in foods interfered with the utilization of isoleucine and lysine. The amino acid score is indicative of the maximum percentage of protein that may be retained for growth, these results coincide with the hypothesis proposed by Garcia and Valverde [19]. The PCA plot Figure 4A clearly showed that no correlation was observed between chlorophyll-a, SST and any of the amino acid indices. However, SST showed similarity with TArAA and NEAA, while chlorophyll-a showed similarity with TEAA along the SE coast Figure 4B. Apparently, chlorophyll-a showed similarity with protein content, TEAA and EAA/NEAA along SW coast Figure 4B. During pre-monsoon along SW coast chlorophyll-a showed high similarity with TNEAA and TSAA.

The high macro minerals in the SE coast suggest that the samples from this coast could be used as good sources of these minerals. The variations recorded in the concentration of the different mineral components in the fish examined could have been as a result of the rate in which these components are available in the water body [20], and the ability of the fish to absorb and convert the essential minerals from the diet or the water bodies where they live. Erkan and Ozden [21] also reported significantly higher values of K content compared to the current findings (158 – 310 mg/100g); at the mean average of 459.7 mg/100g (sea bass) and 393.8 mg/100g (sea bream). Both Na and K are required to maintain osmotic balance of body fluid and the pH of the body regulate muscle and nerve irritability, control glucose absorption and enhance normal retention of protein during growth. A high Na/K ratio observed during post-monsoon in both SW and SE coasts seems to be important because physiological and epidemiological data suggest that a high Na/K ratio intake can be associated with an increased risk of developing high blood pressure and cardiovascular diseases. Phosphorus has been generally associated with the phospholipid content and the presence of phosphoprotein. The high content of Ca and phosphorus were observed in all samples due to the presence of the bones present in them. Food is considered "good" if the Ca/P ratio ≥ 1.0 and "poor" if the ratio ≤ 0.5 while Ca/P ratio ≥ 2.0 helps to increase the absorption of calcium on the small intestine. Oil sardines from both SW and SE coasts during monsoon and post-monsoon seasons would help to increase the absorption of calcium in the small intestine.

Most of the micro minerals were found in higher concentrations in oil sardines, including those important as enzyme substrate activators (Mn and Zn) and as metalloenyzme (Fe). Zn was the most abundant micro element followed by Fe in sardines studied. These discrepancies might be explained in part by seasonal changes in metal concentrations, or the different stages of maturity of the specimens and differences in the annual reproductive cycle of the specimens [22]. In addition, differences in the metal concentrations of the surrounding seawater could influence the metal levels. Increased dietary intake of selenium has been linked to protection against various cancers [23]. Saadettin et al. [24] reported that the most abundant microelements in fish were Zn and Fe followed by Cu with the remaining elements present in amounts below toxic levels. The mean weight and length of the samples (data not shown) varied significantly during sampling periods, which may influence the mineral and trace element concentrations. The content of Mn in all samples was found to be lower than the permissible limit set by FAO/WHO [25], 5.4 ppm or 540 µg/100 g food. Similarly, the oil sardines collected from both coasts contained Zn lower than the limit set by FAO/WHO [25] (150 ppm or 15000 µg/100g). The Se content in oil sardine obtained in oil sardines were significantly higher than cereals (<10 µg/100g), fruits and vegetables (<10 µg/100g) [26]. The abundance of Fe, Mn and Zn among the samples collected from the SW coasts was likely to be due to 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.

Sardine lipid is a rich source of fat soluble vitamins including A, D, E and K which must be taken on a regular basis because of their key roles in human health and metabolism. 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. Vitamin D is essential for the maintenance of normal blood levels of Ca and phosphate [27]. Vitamin E acts as an antioxidant against peroxidation of fatty acid contained in the cellular and sub cellular membrane phospho lipids leading to the formation of phenoxy free radicals. These free radicals formed may react with vitamin C to regenerate tocopherol. 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 [28]. Vitamin K plays an important role in blood clotting and bone metabolism pertaining to the prevention of osteoporosis and carotid artery elasticity.

The present work has elucidated more on the importance of *Sardinella longiceps* as good sources of protein, amino acids, minerals and vitamins. The present study also indicated a reasonably good ratio of essential to nonessential amino acids for sardines from SE coast, and therefore it can be concluded that they are excellent sources of well balanced proteins with high-biological value, and are therefore called as complete proteins. The variation of the protein content is due to the planktonic feed and to climatic changes in the year which influence the general biochemical composition of the fish. Therefore, it is recommended to consume fish and shellfish regularly as it could provide most of minerals needed by the human body.

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List of Abbreviations

TEAA - Total essential amino acids; TNEAA - Total non-essential amino acids; TAA- Total amino acid; TarAA - Total aromatic amino acids; TSAA - Total sulfur containing amino acids; HPLC – High performance liquid chromatography

Statement of Competing Interests

The authors declare that they have no competing interests including any financial, personal or other relationships with other people or organizations that could inappropriately influence, or be perceived to influence, the present work.

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