

Inter-annual variability and seasonal dynamics in lipid signatures of *Leiognathus splendens* (Cuvier, 1829)

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Article history

Received: 8 January 2014
Received in revised form:
6 February 2014
Accepted: 7 February 2014

Keywords

Silver belly
Leiognathus splendens
Fatty acid
Chlorophyll-a
Atherogenic/
thrombogenicity indices
Hypocholesterolemic/
hypercholesterolemic ratio

Abstract

Seasonal variations (pre-monsoon, monsoon and post-monsoon) of the lipid, fatty acid and total cholesterol profile in edible tissues of silver belly *Leiognathus splendens* collected from the south west (SW) and south east (SE) coasts of India over four years (2008-2011) were investigated in this study. The correlations between the fatty acid compositions with seasonal chlorophyll-a concentration of these years were also evaluated. The lipid levels showed pronounced seasonal fluctuations with the highest values occurring in the post-monsoon season and showed a good correlation with chlorophyll-a concentration. The lipid levels showed pronounced seasonal fluctuations with highest values occurring in post-monsoon season and showed strong correlation with chlorophyll-a concentration. Eicosapentaenoic acid correlated with chlorophyll-a concentration during monsoon in SW coast, and monsoon, post-monsoon in the SE coast. The correlation patterns of fatty acids with chlorophyll-a concentration were also studied. Similarly, the health indices such as atherogenic index, thrombogenicity index, total cholesterol, and hypocholesterolemic/hypercholesterolemic ratio of *Leiognathus splendens* were found to be ideal, which contributed towards its parameters to be qualified as an ideal health food.

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Introduction

Marine foods, especially, marine fish are an important part of the human diet. Fatty acids in fish oil have a very distinctive character compared to fatty acids from other sources. They consist not only the essential fatty acids, but also a significant source of *n*-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA, C20:5 *n*-3) and docosahexanoic acid (DHA, C22:6 *n*-3). Human beings are unable to synthesize these long chain PUFAs in their body at a sufficient level from the essential precursors, α -linolenic acid (ALA) and, therefore, they must obtain these fatty acids from their diet. However, as the PUFAs cannot be synthesized de novo by fish, it must be supplied through the diet. Hence the PUFA composition in fishes is related to the life cycle of the fish and external factors like the fatty acid composition of their food, temperature and salinity (Bandarra *et al.*, 2001; Gockse *et al.*, 2004). The annual and seasonal variations in the lipid and fatty acid composition of several marine organisms were reported earlier (Shirai *et al.*, 2002; Luzia *et al.*, 2003; Gockse *et al.*, 2004).

Silver bellies (pony fishes or slip mouths), especially, *Leiognathus splendens* (Cuvier, 1829); (*Leiognathidae*), are a significant group of finfishes widely distributed in Indo-Pacific waters (Ikejima *et*

al., 2004; Kimura *et al.*, 2005). The southern coast is the most productive zone for silver belly in India, occupied about 85% of the total landings (Nair, 2005). Although, several studies have been conducted on the proximate composition, osteological studies, maturation and spawning in silver bellies along the Indian coast (Jayabalan *et al.*, 1985; Abraham *et al.*, 2011), there are no studies on the lipidic signatures of the *L. splendens*. Hence, in the present study the spatial (south west and south east coasts of India), annual (2008 through 2011) and seasonal (pre-monsoon, monsoon and post-monsoon) variations in lipid, fatty acid and cholesterol profiles of *Leiognathus splendens* keeping in mind the implication of such a variation for pharmaceutical products, food additives, and dietary health supplements. There is general agreement that phytoplanktons are the major source of essential fatty acids in the marine environment and the ratio of typical fatty acids can be used as biomarkers for different classes of phytoplanktons (Alkanani *et al.*, 2007). The ocean color images of satellite which was expected to give information about the relationship between the chlorophyll-a concentration and plankton abundance, were statistically studied with respect to the different lipidic parameters. Hence, the present study was undertaken to fill the gaps in the knowledge on seasonal and annual variation in the nutritional profile viz. fatty acids, of the *Leiognathus*

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splendens from the southern coast of India.

Materials and Methods

Materials

Fresh silver bellies (Figure 1) were collected (1 kg each) from the fishing harbors of Mangalore, Calicut, Cochin (SW coast) and Chennai, Mandapam, Tuticorin (SE coast) during 2008–2011 (Chakraborty *et al.*, 2013; Chakraborty *et al.*, 2014). The samples were collected on the 15th day of each month. In order to obtain information on the seasonal variations, the 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 centres of each coast were pooled and the mean values were used in the present study. Two pools of fish per collection site, each composed of 15–20 specimens of comparable body size were collected within each sampling before being washed in sterile water. The whole fish were then gutted and the edible portion was minced for analyses. Although, age and sex differences in the nutritional composition 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.

Estimation of total lipids

The extractions of the lipids in the edible tissues of *L. splendens* collected from SW and SE coast of India were carried out by the Folch extraction method (Folch *et al.*, 1957) using chloroform: methanol (2:1, v/v; 200 mL). The extracted lipids were determined gravimetrically in triplicate.

Fatty acid profiling by gas-chromatographic analysis

The fatty acid composition of the total lipids in the edible tissue of *L. splendens* from SW and SE coast of India were determined as described elsewhere (Chakraborty *et al.*, 2013; Chakraborty *et al.*, 2014). GLC data were recorded on a Perkin-Elmer (USA) AutoSystem XL gas chromatograph (HP 5890 Series II) connected with a SP 2560 (crossbond 5% diphenyl 95% dimethyl polysiloxane) capillary column (100 m X 0.25 mm i.d., 0.50 µm film thickness, Supelco, Bellefonte, PA) using a flame ionization detector (FID) equipped with a split/splitless injector, which was used in the split (1:15) mode. The GC analyses were accomplished using an oven temperature ramp program: 140°C for 1 min, rising at 30°C / min to 250°C, where it was held for 1.0 min, followed by an increase of 25°C / min to 285°C, where it was held for 2.0 min, until all peaks had appeared. The injector and



Figure 1. Photograph of *L. splendens* collected from SW and SE coasts and sampling sites showing SW coast and SE coast of India.

detector were held at 285 and 290°C, respectively. Nitrogen (ultra high purity > 99.99%) was used as the carrier gas at 25 cm/s flow rate. Hydrogen was used as the carrier gas at a head pressure of 20 psi. The injection volume was 0.02 µL. FAMES were identified by comparison of retention times with known standards (Supelco™ 37 Component FAME Mix, Catalog No. 47885-U). Results were expressed as percent of total fatty acids (% TFA).

Evaluation of fatty acid based nutritional indices

The different ratios of fatty acid indicating nutritional values of oil sardines viz., *n-3/n-6*, DHA/EPA, PUFA/SFA and LA/ALA were calculated in order to allow comparisons with the United Kingdom Department of Health recommendations (HMSO, 2001). The indices of atherogenicity (AI) and thrombogenicity (TI) (Ulbricht and Southgate, 1991; Barrento *et al.*, 2010) have been calculated as: $AI = (4 * C14:0 + C18:0 + C16:0) / (MUFA + n-3 PUFA + n-6 PUFA)$, where MUFA means monounsaturated fatty acids and PUFA means polyunsaturated fatty acids; $TI = (C14:0 + C18:0 + C16:0) / [(0.5 * MUFA) + (0.5 * n-3 PUFA) + (3 * n-3 PUFA) + (n-3 PUFA + n-6 PUFA)]$. The hypocholesterolaemic/hypercholesterolaemic (HH) ratio were determined as, $HH = (C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C14:0 + C16:0)$ (Santos-Silva *et al.*, 2002).

Total cholesterol content

The total cholesterol content in the edible tissue of *L. splendens* were determined spectrophotometrically (Varian Cary, USA) as described elsewhere (Wanasundara and Shahidi, 1999) with suitable modification using o-phthalaldehyde (50 mg dL⁻¹ in glacial HOAc). The cholesterol content of the sample was calculated from the standard curve of cholesterol, and expressed as mg/ 100 g wet tissue.

Chlorophyll-a concentration in south west and south east coasts of India

The chlorophyll-a concentrations were

derived from global 9-km monthly mean SeaWiFS (Seaviewing 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/m³ (Chakraborty et al., 2013).

Statistical analysis

Statistical evaluation was carried out with the Statistical Programme 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 biochemical compositions of samples collected was analyzed. The level of significance for all analyses was $p < 0.05$.

Results and Discussion

Seasonal and inter-annual variability in chlorophyll-a concentration along the southwest and southeast coast of India

The variance in the spatial distribution of chlorophyll-a during 2008-2011 with respect to three seasons determined in our earlier study (Chakraborty et al., 2013; Chakraborty et al., 2014) showed that chlorophyll-a showed relatively low values in pre-monsoon (4-year pre-monsoon average 0.3 ± 0.02 mg/m³), reached monsoon maxima (1.2 ± 0.34 mg/m³) and subsequently decreased throughout post-monsoon season (0.5 ± 0.07 mg/m³) in the SW coast. In the SE coast a pre-monsoon minima (0.7 ± 0.17 mg/m³) of chlorophyll-a was followed by the monsoon and post-monsoon maxima (~ 0.8 mg/m³).

*Inter-annual and seasonal variability of lipid content in *L. splendens* collected from the south west and south east coast of India*

The present study indicated that there is substantial variation in lipid composition of *L. splendens* collected from the SE and SW coasts, and there were no significant differences ($p < 0.05$) observed over the years (2008 – 2011) (Table 1A and 1B). The four years averaged lipid content in SE coast was significantly lower during the pre-monsoon season (3.9%; $p < 0.05$). During the warm climate, as in pre-monsoon season, water is poor in nutrients and mineral salts; the fish uses the energy depots in the form of lipids (Keriko et al., 2010) thereby resulting in a significant reduction of lipid content during the pre-monsoon season at the SE coast. However, at the SW coast significantly higher lipid content (8.3%, p

> 0.05) during the pre-monsoon season might be due to the biochemical synthesis of lipid reserves for egg production. The chlorophyll-a concentration during post-monsoon showed a good correlation with the lipid content (8.4 & 6.3%, respectively, $p > 0.05$) at the SW and SE coasts (Figure 2 A and B, respectively; $r^2 = 0.946$ & 0.962 , respectively).

*Inter-annual and seasonal variability of fatty acid content in *L. splendens* collected from the south west and south east coast of India*

The inter-annual and seasonal variability of fatty acid composition in *L. splendens* collected from the south west and south east coast of India have been demonstrated in Table 1A and 1B, respectively. The prevalence of SFAs (34.3 and 37.7%) over the MUFAs (30.9 and 26.6%) and PUFAs (24.6 and 26.3%) at the samples from the SW and SE coasts, respectively, was observed, during the present study. SFA with their high caloric content is primarily used as a source or the storage form of energy, and therefore, their concentration increased during the periods of enhanced feeding activity (Shirai et al., 2002). The samples from the SE coast exhibited comparatively higher content of SFAs than those from the SW coast. This could be due to the natural variation in the accumulation of fatty acids and the differences in environmental conditions. No inter-annual variability was observed for the samples collected during a particular season in the case of SFA content along both the coasts. Among the SW coast, four years averaged SFA content was found to be maximum during the monsoon season (34.9%). A significantly higher SFA content was observed during the monsoon (40.1%, $p < 0.05$), when compared to other seasons. This might be due to the enhanced feeding activity during the monsoon season, which was evident from the high chlorophyll-a concentration during the period. The predominant SFA (<18.6% and <19.6% in SW and SE coast samples, respectively) in *L. splendens* was palmitic acid (C16:0), which was noted as the predominant source of potential metabolic energy for growing fish (Henderson et al., 1984). Earlier reports also showed C16:0 as the predominant SFA in other species of the family *Leiognathidae* including *L. splendens* (Chandrani et al., 2012).

A significantly high MUFA content was observed in the samples collected from the SW coast (28.7 - 33.8%) than the SE coast (23.1 - 33.8%) ($p < 0.05$). There were no significant inter-annual differences observed for MUFA ($p > 0.05$) over the experimental period spanning from 2008 – 2011. The mean MUFA content in the SW coast samples did not show significant variation over studied seasons ($p > 0.05$).

Table 1A. Fatty acid composition (%TFA) of *L. splendens* collected from SW coast of India during 2008-11 in three different seasons (Pre-monsoon, Monsoon and Post-monsoon).

	Pre-monsoon			Monsoon				Post-monsoon				
	2008	2009	2010	2008	2009	2010	2011	2008	2009	2010	2011	
Lipid (%)	8.49±0.46 ^a	8.19±0.27 ^a	7.41±0.55 ^a	9.3±0.02 ^a	4.9±0.02 ^b	3.7±0.01 ^b	3.2±0.51 ^b	4.5±0.46 ^b	9.1±0.44 ^a	5.15±0.33 ^{ab}	9.53±0.12 ^a	9.74±0.23 ^a
Saturated Fatty acids												
C12:0	0.14±0.02 ^a	0.2±0.03 ^a	0.21±0.03 ^a	0.14±0.02 ^a	0.28±0.04 ^{ab}	0.46±0.07 ^b	0.23±0.03 ^a	0.23±0.03 ^a	0.1±0.01 ^a	0.13±0.02 ^a	0.13±0.02 ^a	0.15±0.02 ^a
C14:0	3.41±0.49 ^a	3.76±0.54 ^a	3.89±0.56 ^a	3.61±0.52 ^a	2.8±0.4 ^a	3.66±0.52 ^a	4.5±0.64 ^a	3.64±0.52 ^a	3.18±0.46 ^a	3.62±0.52 ^a	3.32±0.47 ^a	3.84±0.55 ^a
C15:0	1.02±0.15 ^a	1.11±0.16 ^a	1.29±0.18 ^{ac}	0.83±0.12 ^b	1.16±0.17 ^a	1.51±0.22 ^c	1.34±0.19 ^c	1.07±0.15 ^a	0.98±0.14 ^b	1.38±0.2 ^c	0.94±0.13 ^b	1.01±0.14 ^a
C16:0	16.7±0.9 ^a	17.6±1.1 ^a	16.6±1.2 ^a	18.3±1.31 ^a	16.87±1.14 ^a	17.5±1.04 ^a	17.3±1.1 ^a	18.5±1.9 ^a	16.59±1.4 ^a	18.17±1.2 ^a	18.6±1.1 ^a	17.6±1.5 ^a
C17:0	0.6±0.09 ^a	0.56±0.08 ^a	0.58±0.08 ^a	0.85±0.12 ^{ab}	1.36±0.19 ^c	1.24±0.03 ^c	0.86±0.01 ^{ab}	0.98±0.03 ^b	1.1±0.16 ^b	1.26±0.18 ^c	0.83±0.12 ^{ab}	0.68±0.1 ^a
C18:0	9.8±0.19 ^a	8.9±0.02 ^a	8.8±0.12 ^a	9.1±0.25 ^a	9.6±0.24 ^a	8.89±0.58 ^a	10±0.55 ^a	9.19±0.25 ^a	7.67±0.16 ^b	10±0.87 ^a	9.02±0.42 ^a	9.6±0.24 ^a
C20:0	0.91±0.13 ^a	0.79±0.11 ^a	0.78±0.11 ^a	0.7±0.1 ^a	0.89±0.13 ^a	0.84±0.12 ^a	0.72±0.1 ^a	0.67±0.1 ^a	0.85±0.12 ^a	0.82±0.12 ^a	0.79±0.11 ^a	0.84±0.12 ^a
C22:0	0.9±0.13 ^a	0.85±0.12 ^a	0.69±0.1 ^{ab}	0.31±0.04 ^b	0.28±0.04 ^b	0.47±0.07 ^b	0.37±0.05 ^b	0.29±0.04 ^b	0.43±0.06 ^b	0.92±0.13 ^a	0.87±0.12 ^a	0.84±0.12 ^a
C24:0	0.36±0.05 ^a	0.31±0.04 ^a	0.29±0.04 ^a	0.14±0.02 ^a	0.63±0.09 ^b	0.48±0.07 ^{ab}	0.22±0.03 ^a	0.39±0.06 ^a	0.45±0.06 ^{ab}	0.42±0.06 ^{ab}	0.35±0.05 ^a	0.3±0.04 ^a
ΣSFA ^a	33.84±3.1 ^a	34.08±2.5 ^a	33.13±2.1 ^a	33.98±2.0 ^a	33.87±1.9 ^a	35.05±3.2 ^a	35.54±2.8 ^a	34.96±2.15 ^a	31.35±2.8 ^a	36.72±1.45 ^a	34.85±1.9 ^a	34.86±3.14 ^a
Monounsaturated Fatty acids												
C14:1n-7	0.05±0.01 ^c	0.2±0.03 ^a	0.21±0.03 ^a	ND	ND	0.11±0.02 ^b	0.1±0.01 ^b	0.1±0.01 ^b	0.22±0.03 ^a	0.25±0.04 ^a	0.29±0.04 ^a	0.25±0.04 ^a
C15:1n-7	0.1±0.01 ^a	0.19±0.03 ^a	0.11±0.02 ^a	ND	ND	0.08±0.01 ^a	0.09±0.01 ^a	0.12±0.02 ^a	0.15±0.02 ^{ab}	0.15±0.02 ^{ab}	0.16±0.02 ^{ab}	0.16±0.02 ^{ab}
C16:1n-7	6.46±0.92 ^a	6.76±0.97 ^a	6.59±0.94 ^a	6.56±0.94 ^a	5.93±0.85 ^a	5.07±0.72 ^a	6.45±0.92 ^a	6.79±0.97 ^a	7.41±1.06 ^a	6.49±0.94 ^a	6.55±0.94 ^a	6.6±0.94 ^a
C18:1n-7	0.32±0.05 ^a	0.45±0.06 ^a	0.42±0.06 ^a	ND	ND	0.42±0.06 ^a	0.41±0.06 ^a	0.42±0.06 ^a	0.54±0.08 ^a	0.54±0.08 ^a	0.39±0.06 ^a	0.32±0.05 ^a
C18:1n-9	17.92±2.56 ^a	17.93±2.56 ^a	18.12±2.59 ^a	20.92±2.99 ^a	22.18±3.17 ^a	17.93±2.56 ^a	17.2±2.46 ^a	20.62±9.5 ^a	20.32±2.91 ^a	17.96±2.57 ^a	19.15±2.74 ^a	17.13±2.45 ^a
C20:1n-9	0.87±0.12 ^a	0.82±0.12 ^a	0.83±0.12 ^a	0.57±0.08 ^{ab}	0.3±0.04 ^b	0.67±0.1 ^{ab}	1.05±0.15 ^c	0.82±0.12 ^a	0.04±0.01 ^d	0.11±0.02 ^d	0.37±0.05 ^b	0.8±0.12 ^a
C22:1n-9	3.89±0.56 ^a	4.04±0.58 ^a	3.73±0.53 ^a	ND	ND	3.18±0.46 ^a	3.1±0.44 ^a	3.21±0.46 ^a	4.44±0.63 ^a	4.19±0.63 ^a	3.74±0.53 ^a	3.65±0.52 ^a
C24:1n-9	0.49±0.07 ^a	0.43±0.06 ^a	0.45±0.06 ^a	1.42±0.2 ^b	5.01±0.72 ^c	3.45±0.49 ^c	0.38±0.05 ^b	0.62±0.09 ^a	0.75±0.11 ^a	0.7±0.1 ^a	0.57±0.08 ^a	0.46±0.07 ^a
ΣMUFA ^b	30.08±2.9 ^a	30.79±2.5 ^a	30.44±2.55 ^a	29.45±2.6 ^a	33.34±2.3 ^a	30.88±2.3 ^a	28.78±1.3 ^a	32.68±2.8 ^a	33.85±3.3 ^a	30.33±3.6 ^a	31.17±2.9 ^a	29.35±1.55 ^a
Polyunsaturated Fatty acids												
C16:2n-4	0.31±0.04 ^{ab}	0.41±0.06 ^{ab}	0.46±0.07 ^{ab}	ND	0.6±0.09 ^b	ND	0.61±0.09 ^b	ND	ND	0.14±0.02 ^a	0.2±0.03 ^a	0.33±0.05 ^{ab}
C16:3n-4	0.38±0.05 ^a	0.38±0.05 ^a	0.24±0.03 ^b	ND	0.28±0.04 ^a	ND	0.24±0.03 ^b	ND	ND	0.18±0.03 ^b	0.25±0.04 ^a	0.37±0.05 ^a
C18:2n-6	1.34±0.19 ^a	1.44±0.21 ^{ab}	1.38±0.2 ^a	1.34±0.19 ^a	1.53±0.03 ^{ab}	1.25±0.11 ^a	1.68±0.24 ^{ab}	1.25±0.11 ^a	1.13±0.16 ^a	1.44±0.21 ^{ab}	1.24±0.18 ^{ac}	1.35±0.19 ^a
C18:3n-6	0.66±0.01 ^a	0.83±0.05 ^a	1.18±0.11 ^{ab}	1.86±0.14 ^b	1.77±0.01 ^b	1.12±0.09 ^{ab}	1.84±0.05 ^b	1.12±0.09 ^{ab}	1.7±0.02 ^{ab}	1.19±0.01 ^{ab}	1.02±0.04 ^a	0.68±0.04 ^a
C18:3n-3	0.73±0.1 ^a	0.75±0.11 ^a	0.73±0.1 ^a	1.75±0.25 ^b	2.19±0.31 ^b	2.54±0.36 ^b	1.13±0.16 ^a	2.54±0.36 ^b	0.68±0.1 ^a	0.33±0.05 ^c	0.54±0.08 ^{ac}	0.74±0.1 ^a
C18:4n-3	0.1±0.01 ^a	ND	ND	0.23±0.03 ^b	0.37±0.05 ^b	0.28±0.04 ^b	0.26±0.04 ^b	0.28±0.04 ^b	0.14±0.02 ^a	0.08±0.01 ^a	0.13±0.02 ^a	0.1±0.01 ^a
C20:2n-6	0.84±0.12 ^a	0.92±0.13 ^a	0.83±0.12 ^a	0.57±0.08 ^{ac}	0.63±0.09 ^{ac}	0.28±0.04 ^b	0.20±0.15 ^a	0.28±0.04 ^b	0.9±0.13 ^a	0.28±0.12 ^a	0.79±0.11 ^a	0.84±0.12 ^a
C20:3n-6	0.3±0.04 ^a	0.24±0.03 ^a	0.27±0.04 ^a	0.53±0.08 ^a	0.52±0.07 ^a	0.88±0.13 ^b	0.39±0.06 ^a	0.88±0.13 ^b	0.25±0.04 ^a	0.31±0.04 ^a	0.26±0.04 ^a	0.26±0.04 ^a
C20:4n-6	0.32±0.05 ^a	0.44±0.06 ^a	0.88±0.13 ^a	0.59±0.03 ^a	2.67±0.01 ^b	2.67±0.01 ^b	1.88±0.03 ^b	2.67±0.01 ^b	0.42±0.02 ^a	0.39±0.02 ^a	0.69±0.03 ^a	0.35±0.02 ^a
C20:5n-3	10.61±0.93 ^a	10.39±0.55 ^a	9.36±0.75 ^a	9.33±0.58 ^a	9.9±1.1 ^a	8.58±1.02 ^a	9.26±0.92 ^a	8.58±1.02 ^a	11.4±1.02 ^a	9.25±1.1 ^a	9.83±0.98 ^a	9.82±0.95 ^a
C22:5n-3	0.94±0.13 ^a	0.81±0.12 ^a	0.95±0.14 ^a	1.66±0.24 ^{ac}	1.23±0.18 ^a	2.43±0.35 ^a	1.44±0.2 ^a	2.43±0.35 ^a	2.37±0.34 ^{bc}	1.23±0.18 ^a	1.67±0.24 ^{ac}	1.02±0.15 ^a
C22:6n-3	7.97±0.54 ^a	5.46±0.42 ^b	5.45±0.23 ^b	7.34±0.26 ^a	6.1±0.65 ^{ab}	6.05±0.55 ^{ab}	7.43±0.25 ^a	6.05±0.55 ^{ab}	6.58±0.28 ^{ab}	7.38±0.19 ^a	6.24±0.28 ^{ab}	6.73±0.28 ^{ab}
ΣPUFA ^c	24.5±1.26 ^a	21.99±0.95 ^a	21.73±1.93 ^a	25.2±1.24 ^{ab}	27.08±1.25 ^{ab}	26.08±1.43 ^{ab}	27.14±1.45 ^b	26.08±1.43 ^{ab}	25.57±1.99 ^{ab}	22.77±1.52 ^a	22.56±1.24 ^a	22.55±1.21 ^a

Data are expressed as mean ± standard deviation of three replicates. ^aΣSFA Total saturated fatty acids, ^bΣMUFA Total monounsaturated fatty acids, ^cΣPUFA Total polyunsaturated fatty acids. Means with different superscripts (a, b, c, d) in the same row indicates a statistical difference (p < 0.05). ND: not detected.

Table 1B. Fatty acid composition (%TFA) of *L. splendens* collected from SE coast of India during 2008-11 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
Lipid (%)	4.75±0.46 ^a	4.82±0.24 ^a	5.22±0.40 ^a	4.14±0.41 ^a	5.14±0.46 ^a	3.76±0.33 ^a	3.62±0.22 ^a	2.51±0.12 ^a	6.67±0.89 ^a	5.07±0.23 ^a	7.09±0.54 ^a	6.51±0.12 ^a
Saturated Fatty acids												
C12:0	0.1±0.01 ^a	0.41±0.06 ^{ab}	0.32±0.05 ^{ab}	0.57±0.08 ^{ab}	0.72±0.1 ^a	0.24±0.03 ^a	0.24±0.03 ^a	0.2±0.03 ^a	0.13±0.02 ^a	0.15±0.02 ^a	0.13±0.02 ^a	0.13±0.02 ^a
C14:0	3.18±0.46 ^a	3.36±0.48 ^a	4.48±0.64 ^a	3.69±0.53 ^a	2.59±0.37 ^a	3.74±0.53 ^a	4.91±0.73 ^a	5.24±0.75 ^a	4.05±0.58 ^a	4.5±0.64 ^a	4.08±0.58 ^a	4.09±0.58 ^a
C15:0	0.98±0.14 ^a	1.51±0.22 ^a	2.22±0.32 ^{ab}	3.27±0.47 ^b	2.58±0.37 ^{ab}	2.5±0.36 ^{ab}	1.6±0.23 ^a	1.36±0.19 ^a	1.48±0.21 ^a	1.45±0.21 ^a	1.59±0.23 ^a	1.51±0.22 ^a
C16:0	19.55±3.3 ^a	18.13±0.3 ^a	18.47±0.3 ^a	17.75±0.3 ^a	18.88±0.3 ^a	19.48±0.3 ^a	18.42±0.3 ^a	19.57±0.3 ^a	18.83±0.3 ^a	19.62±0.3 ^a	18.26±0.3 ^a	19.05±0.3 ^a
C17:0	1.1±0.16 ^{ab}	1.88±0.27 ^b	2.03±0.29 ^b	2.41±0.34 ^b	2.09±0.3 ^{ab}	2.01±0.29 ^b	1.77±0.25 ^b	1.11±0.16 ^{ab}	0.58±0.08 ^a	0.49±0.07 ^a	0.48±0.07 ^a	0.54±0.08 ^a
C18:0	7.67±0.53 ^a	7.63±0.23 ^a	7.9±0.32 ^a	7.55±0.43 ^a	11.4±0.55 ^{ab}	11.6±0.37 ^{ab}	10.5±0.13 ^b	10.9±0.37 ^b	11±0.16 ^b	10.2±0.25 ^b	10.6±0.83 ^b	10.4±0.83 ^b
C20:0	0.85±0.12 ^a	0.72±0.1 ^a	0.59±0.08 ^{ab}	0.39±0.06 ^b	0.56±0.08 ^{ab}	0.66±0.09 ^{ab}	0.83±0.12 ^a	0.82±0.12 ^a	0.69±0.1 ^a	0.8±0.11 ^a	0.8±0.11 ^a	0.74±0.11 ^a
C22:0	0.43±0.06 ^a	0.46±0.07 ^a	0.53±0.08 ^a	0.55±0.08 ^a	0.34±0.05 ^b	0.38±0.05 ^b	0.4±0.06 ^{ab}	0.41±0.06 ^{ab}	0.27±0.04 ^b	0.33±0.05 ^{ab}	0.39±0.06 ^{ab}	0.32±0.05 ^{ab}
C24:0	0.45±0.06 ^a	1.65±0.24 ^d	0.28±0.04 ^b	0.66±0.09 ^a	0.93±0.13 ^{ad}	0.58±0.08 ^a	0.15±0.02 ^c	0.19±0.03 ^c	0.28±0.04 ^b	0.28±0.04 ^b	0.21±0.03 ^b	0.25±0.04 ^b
ΣSFA ^a	34.31±1.25 ^a	35.75±2.3 ^a	36.82±0.25 ^a	36.84±2.54 ^a	40.09±4 ^a	41.19±2.15 ^b	39.62±3.26 ^b	39.4±2.89 ^b	37.21±0.92 ^{ab}	38.62±3.44 ^{ab}	36.14±3.15 ^a	37.23±3.14 ^{ab}
Monounsaturated Fatty acids												
C14:1n-7	0.22±0.03 ^a	0.15±0.02 ^c	0.27±0.04 ^a	0.44±0.06 ^b	0.23±0.03 ^a	0.22±0.03 ^a	0.22±0.03 ^a	0.3±0.04 ^a	0.27±0.04 ^a	0.34±0.05 ^a		

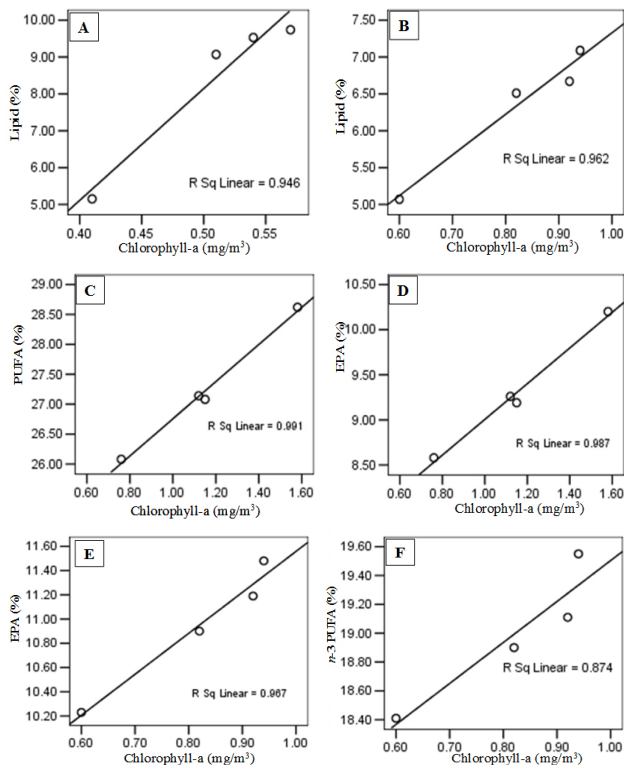


Figure 2. The correlation between (A) chlorophyll-a & lipid content during post-monsoon along SW coast, (B) chlorophyll-a & lipid content during post-monsoon along SE coast, (C) chlorophyll-a & PUFA content during monsoon along SW coast, (D) chlorophyll-a & EPA content during monsoon along SW coast, (E) chlorophyll-a & EPA content during monsoon along SE coast and (F) chlorophyll-a & *n*-3 PUFA content during monsoon along SE coast.

and 4.7 - 8.6%, respectively) at the SW and SE coasts throughout the studied period ($p < 0.05$). A good correlation of EPA with chlorophyll-a concentration (Figure 2D; $r^2 = 0.987$ in SW coast and Figure 2E $r^2 = 0.967$ in SE coast) was observed during monsoon season. It is of note that at the SW coast, the increase of chlorophyll-a concentration during the monsoon season provides an adequate food supply for fish, which is coincident with the higher PUFA content during the monsoon (27.2%).

The total *n*-3 PUFAs were generally higher than those of *n*-6 PUFA over the studied periods. During the post-monsoon season, a strong correlation was observed for *n*-3 PUFA with chlorophyll-a concentration in the SE coast (Figure 2F; $r^2 = 0.874$). However, the significantly lower content of PUFA ($p < 0.05$) was discernible during the pre-monsoon and post-monsoon seasons (23.4 and 23.3%, respectively), and these results can be corroborated with the low chlorophyll-a concentration in these seasons ($r^2 = 0.991$). In the SE coast, significantly lower PUFA content was observed during the post-monsoon (23.6%) than the preceding seasons (28.8

and 26.6%) mainly due to the decrease in *n*-6 PUFA such as arachidonic acid (0.5%) and *n*-3 PUFA α -linolenic acid (0.2%). The *n*-3 PUFA content perceived a monsoon maximum (four years average - 20%) in the samples harvested from the SW coast, where the chlorophyll-a concentration recorded high values, and pre-monsoon minima (four years average - 18.6%) where the chlorophyll-a concentration noted low values. However, a pre-monsoon maxima (21.4%) and post-monsoon minima (19%) in *n*-3 PUFA content were observed in the samples from the SE coast. The *n*-6 PUFA content observed a monsoon maximum (6.8%) in *L. splendens* collected from the SW coast and pre-monsoon maxima (6.9%) in SE coast.

Inter-annual and seasonal variability of nutritional indices in L. splendens collected from the south west and south east coasts of India

The *n*-3/*n*-6 ratio is a marker of biomedical significance for fish oils and could be an index of biomedical application. These ratios amounted to be lower than 5.8 in *L. splendens* from the SW coast, and lower 4.8 in the SE coast. Although insignificant, the difference in the *n*-3/*n*-6 ratio between different seasons in the present study may be explained by the large variability of the lipid content of the fish, which depends on the species, period of the year, reproduction period, as well as the fatty acid composition of the diet (Shirai *et al.*, 2002). The *n*-6/*n*-3 ratio has been suggested to be a useful indicator for comparing the relative nutritional value of a given fish. According to the UK Department of Health, a ratio within 0.2 - 1.5 would constitute a healthy human diet (HMSO, 2001), and values higher than 1.5 would be risky, and may promote cardiovascular diseases. In the present study the ratio found to be within the recommended range in the fish obtained from the SW and SE coasts (<0.37 and <0.5, respectively). The PUFA/SFA ratio used to indicate the nutritional quality of lipid and the recommended ratio is 0.4 - 1 (Jiménez-Colmenero, 2007). In the present study, the ratio was found to be lower than 0.7 and lower than 0.9, respectively in SW and SE coast samples. The DHA/EPA ratio noted to be less (<0.8) for the samples collected from both the coasts.

In human nutrition, linoleic acid (C18:2*n*-6) and linolenic acid (C18:3*n*-3) are regarded as essential since they cannot be synthesized in the human body from any other fatty acids provided in our diet. The human metabolic pathways can synthesize the PUFAs from the two precursors the α -linolenic acid (ALA, C18:3*n*-3) and linoleic acid (LA, C18:2*n*-6) by two different enzymes elongase and desaturase (Garaffo

Table 2. Nutritional composition (% TFA) of *L. splendens* collected from south east coast of India during 2008-'11 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
Southwest coast												
$\Sigma n-3$	20.35±1.25 ^a	17.41±1.24 ^b	16.49±1.13 ^b	20.31±2.1 ^a	21.7±1.65 ^a	19.88±1.99 ^a	19.48±1.22 ^b	19.08±1.47 ^b	21.17±1 ^a	18.27±1.25 ^a	18.41±1.22 ^b	18.37±1.85 ^b
$\Sigma n-6$	3.46±1 ^a	3.79±1.75 ^a	4.54±1.64 ^a	4.89±1.45 ^a	6.92±1.17 ^b	6.2±1.87 ^{ab}	6.81±1.27 ^b	7.12±1.28 ^b	4.4±1.24 ^a	4.18±1.45 ^a	3.7±1.87 ^a	3.48±1.22 ^a
$n-3/n-6$	5.88±0.42 ^a	4.59±0.33 ^a	3.63±0.25 ^{ab}	4.15±0.26 ^{ab}	3.14±0.26 ^b	3.21±0.54 ^a	2.86±0.23 ^b	2.68±0.42 ^b	4.81±0.55 ^{ab}	4.37±0.65 ^{ab}	4.98±0.25 ^a	5.28±0.25 ^a
$n-6/n-3$	0.17±0.02 ^a	0.22±0.012 ^a	0.28±0.014 ^a	0.24±0.016 ^a	0.32±0.016 ^{ab}	0.31±0.02 ^{ab}	0.35±0.014 ^b	0.37±0.012 ^b	0.21±0.015 ^a	0.23±0.012 ^a	0.2±0.018 ^a	0.19±0.01 ^a
C22:6n-3/ C20:5n-3	0.75±0.02 ^a	0.53±0.05 ^b	0.58±0.01 ^b	0.79±0.04 ^a	0.71±0.01 ^a	0.71±0.04 ^a	0.8±0.02 ^a	0.66±0.03 ^{ab}	0.58±0.04 ^b	0.8±0.03 ^a	0.63±0.02 ^{ab}	0.69±0.021 ^{ab}
Σ PUFA/ Σ SFA	0.72±0.04 ^a	0.65±0.05 ^a	0.66±0.02 ^a	0.74±0.03 ^a	0.84±0.012 ^a	0.74±0.02 ^a	0.76±0.02 ^a	0.77±0.04 ^a	0.82±0.014 ^a	0.62±0.016 ^a	0.65±0.015 ^a	0.65±0.054 ^a
C18:2n-6/ C18:3n-3	1.84±0.02 ^a	1.92±0.03 ^a	1.89±0.02 ^a	0.77±0.04 ^b	0.69±0.34 ^b	0.49±0.05 ^b	1.49±0.26 ^b	0.7±0.25 ^b	1.66±0.24 ^b	4.36±0.29 ^b	2.3±0.28 ^{bc}	1.93±0.24 ^b
AI	0.74±0.01 ^a	0.8±0.02 ^a	0.8±0.02 ^a	0.77±0.02 ^a	0.61±0.02 ^a	0.7±0.02 ^a	0.8±0.02 ^a	0.7±0.02 ^a	0.6±0.02 ^a	0.81±0.02 ^a	0.77±0.02 ^a	0.83±0.02 ^a
TI	0.36±0.02 ^a	0.41±0.01 ^a	0.41±0.02 ^a	0.38±0.02 ^a	0.33±0.02 ^a	0.37±0.03 ^a	0.4±0.01 ^a	0.4±0.02 ^a	0.3±0.03 ^a	0.4±0.01 ^a	0.4±0.01 ^a	0.4±0.01 ^a
HH ratio	1.98±0.01 ^a	1.74±0.01 ^a	1.8±0.01 ^a	1.96±0.01 ^a	2.4±0.01 ^a	2±0.01 ^a	1.8±0.01 ^a	2±0.01 ^a	2.2±0.02 ^a	1.74±0.01 ^a	1.78±0.01 ^a	1.73±0.01 ^a
Total cholesterol	21.2±0.01 ^a	23.58±0.01 ^a	26.2±0.01 ^a	28.5±0.51 ^a	41.5±0.02 ^a	37.3±0.41 ^{ab}	44±0.02 ^b	41±0.02 ^b	56±0.32 ^b	37±0.22 ^b	58.2±0.32 ^b	51.72±0.12 ^b
Southeast coast												
$\Sigma n-3$	21.17±1.25 ^a	22.6±1.24 ^a	19.32±1.13 ^b	22.37±2.1 ^a	20.43±1.65 ^a	19.22±1.99 ^a	19±1.22 ^b	20.34±1.47 ^b	19.37±1.25 ^b	18.15±1 ^a	19.48±1.85 ^a	18.97±1.22 ^a
$\Sigma n-6$	7.4±1 ^{ab}	9.84±1.75 ^b	4.52±1.64 ^a	5.95±1.45 ^{ab}	4.18±1.17 ^b	9.61±1.87 ^b	4.86±1.27 ^b	6.28±1.28 ^{ab}	4.28±1.45 ^a	4.04±1.24 ^a	4.16±1.22 ^a	4.35±1.87 ^a
$n-3/n-6$	2.86±0.55 ^a	2.3±0.36 ^a	4.27±0.25 ^b	3.76±0.26 ^{ab}	4.89±0.28 ^b	2±0.34 ^a	3.91±0.29 ^{ab}	3.24±0.24 ^a	4.53±0.87 ^b	4.49±0.24 ^b	4.68±0.25 ^b	4.36±0.45 ^b
$n-6/n-3$	0.35±0.02 ^{ab}	0.44±0.012 ^a	0.23±0.014 ^a	0.27±0.016 ^a	0.2±0.011 ^a	0.5±0.015 ^a	0.26±0.013 ^b	0.31±0.012 ^b	0.22±0.017 ^b	0.22±0.017 ^b	0.21±0.01 ^b	0.23±0.018 ^b
C22:6n-3/ C20:5n-3	0.58±0.02 ^a	0.58±0.05 ^a	0.59±0.01 ^a	0.84±0.04 ^a	0.7±0.01 ^{ab}	0.42±0.04 ^a	0.71±0.02 ^{ab}	0.54±0.03 ^a	0.59±0.03 ^a	0.62±0.04 ^a	0.55±0.021 ^a	0.58±0.02 ^a
Σ PUFA/ Σ SFA	0.83±0.04 ^a	0.93±0.05 ^a	0.67±0.02 ^a	0.78±0.03 ^a	0.63±0.021 ^a	0.59±0.02 ^a	0.68±0.05 ^a	0.74±0.04 ^a	0.64±0.041 ^a	0.58±0.055 ^a	0.66±0.054 ^a	0.64±0.021 ^a
C18:2n-6/ C18:3n-3	1.66±0.02 ^a	0.71±0.03 ^b	0.88±0.02 ^a	0.98±0.04 ^a	1.85±0.34 ^a	1.63±0.05 ^a	2.89±0.26 ^{ab}	1.77±0.25 ^b	6.38±0.29 ^b	4.59±0.24 ^b	5.6±0.24 ^b	7.24±0.28 ^b
AI	0.64±0.02 ^a	0.62±0.02 ^a	0.9±0.02 ^a	0.72±0.02 ^a	0.7±0.03 ^a	0.9±0.02 ^a	1±0.02 ^a	1.1±0.02 ^a	1±0.02 ^a	1.01±0.02 ^a	0.91±0.02 ^a	0.96±0.02 ^a
TI	0.35±0.02 ^a	0.32±0.02 ^a	0.4±0.02 ^a	0.33±0.01 ^a	0.42±0.01 ^a	0.46±0.02 ^a	0.4±0.01 ^a	0.4±0.02 ^a	0.4±0.03 ^a	0.5±0.01 ^a	0.42±0.01 ^a	0.45±0.01 ^a
HH ratio	2.02±0.02 ^a	2.14±0.02 ^a	1.53±0.02 ^a	1.89±0.02 ^a	2.4±0.02 ^a	1.5±0.02 ^a	1.4±0.02 ^a	1.4±0.02 ^a	1.4±0.02 ^a	1.5±0.02 ^a	1.57±0.02 ^a	1.48±0.02 ^a
Total cholesterol	26.3±0.02 ^a	21.13±0.22 ^a	26.9±0.12 ^a	28.3±0.42 ^a	36.5±0.44 ^a	33.2±0.62 ^a	39±0.22 ^b	36±0.27 ^b	54±0.35 ^b	48±0.54 ^b	57.1±0.62 ^b	52.89±0.42 ^b

Data are expressed as mean ± standard deviation of three replicates. *n-3* means total *n-3* fatty acids; *n-6* means total *n-6* fatty acids; C20:5n-3 = EPA; C22:6n-3 = DHA; Σ SFA Total saturated fatty acids; Σ PUFA = Total polyunsaturated fatty acids; C18:2n-6 = Linoleic acid; C18:3n-3 = α -linolenic acid; AI = atherogenicity index; TI = Thrombogenicity index; HH ratio = hypocholesterolemic/hypercholesterolemic ratio; Total cholesterol content is expressed in mg/100 g edible portion. Different superscripts (a, b, c, d) in the same row indicate statistical difference ($p < 0.05$).

et al., 2011). Vertebrates lack $\Delta 12$ and $\Delta 15$ (*n-3*) fatty acid desaturases, and therefore, cannot produce LA and ALA from oleic acid (Miller *et al.*, 2007). In the present investigation the LA/ALA ratio observed to be lower in SW coast, especially during monsoon season (~0.8).

In the present study, atherogenicity index (AI) and thrombogenicity index (TI) (Table 2) of *L. splendens* were taken into account to understand the importance of this marine fish species as the candidate health food. These parameters are of importance because they attest the importance of the food items for their utility to protect the human body from atherosclerosis and platelet aggregation due to the anti-atherogenic and anti-thrombogenic action of the *n-3* PUFAs (Ulbricht and Southgate, 1991). The AI and TI values were found to be lower than 0.83 and 0.41 in the samples collected from the SW coast, whereas these indices were found to be lower than 1 and 0.47, respectively in the samples from the SE coast. These values were comparable with rabbit meat (0.70 and 0.99), pigeon meat (0.41 and 0.94) and poultry meat (0.49 and 0.88) (Dal Bosco *et al.*, 2012). It is of note that the HH (Table 2) ratio is directly related to the cholesterol metabolism, and a higher value of this ratio is prudent from a nutritional point of view (Ramos-Filho *et al.*, 2010). In *L. splendens* the HH ratio perceived to be higher during the monsoon season (2.0) in the SW coast and during pre-monsoon (1.9) in the SE coast.

Inter-annual and seasonal variation of total cholesterol content in L. splendens collected from the

south west and the south east coast

Cholesterol forms the building blocks of several compounds such as bile, sex hormones, adrenal hormones, and vitamin D. The total cholesterol level in *L. splendens* ranged 21.1 – 58.1 mg% in the SW coast and 21.1 – 57.1 mg% in the SE coast (Table 2). The four years mean cholesterol during the post-monsoon season was found to be significantly higher in the samples collected from the SW and SE coasts (55.7 and 53.1 mg%, respectively). It is significant to notice that the cholesterol content in the *L. splendens* was found to be lower than the conventional food items such as beef (84 mg%), pork (79 mg%), chicken (85 mg%), cheese (105 mg%) and eggs (424 mg%) (USDA, 1998). The daily intake limit of cholesterol as recommended by the National Cholesterol Education Program is lower than 300 mg/100 g (NCEP, 2002). These results prove that *L. splendens* is a preferred fish species for good human health, relating to prevention of atherosclerotic coronary disease. A negative relationship ($r^2 = 0.573$) between the cholesterol and PUFA contents and PUFA to SFA ratio, especially during monsoon also supports the fact that dietary PUFA-fat modulate the levels of cholesterol in proportion to the increase in the ratio of PUFA to SFA, and show that the changes in the cholesterol reflected in the changes in the fatty acid composition.

Conclusion

The present study showed that the nutritional

quality indices with respect to the lipid and fatty acids were more favorable in *Leiognathus splendens* harvested from the SW coast as compared to those from the SE coast. *L. splendens* is a rich source of long chain *n*-3 polyunsaturated fatty acids such as EPA and DHA, with acceptable ratios of *n*-3/*n*-6 and PUFA/SFA is conforming to its optimal nutritional qualities. Silver belly has been demonstrated to be a candidate marine fish species and valued candidate variety for human nutrition due to the adherence towards ideal nutritional qualities. A high hypocholesterolemic-hypercholesterolemic ratio with lower atherogenic and thrombogenicity indices of silver bellies make this a preferred health food for human consumption.

Acknowledgements

The authors are thankful to Indian Council of Agricultural Research, New Delhi for providing necessary facilities and encouragements to carry out the work under ICAR Outreach Activity-3 on "Nutrient profiling of fish as a food for health and dietary component". The authors thank the Director, Central Marine Fisheries Research Institute, for his guidance and support. Thanks are due to the Head, Marine Biotechnology Division, Central Marine Fisheries Research Institute for facilitating the research activity.

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