



Note

Seasonal variation of fatty acid profile in edible tissue of deep-sea whip lobster *Puerulus sewelli* (Decapoda, Palinuridae)

KAJAL CHAKRABORTY, SOUMYA KRISHNAN, REKHA DEVI CHAKRABORTY*,
ASWATHY ELIZABETH MANI AND P. VIJAYAGOPAL

Marine Biotechnology, Fish Nutrition and Health Division, ICAR-Central Marine Fisheries Research Institute, Ernakulam North P. O., P.B. No. 1603, Kochi - 682 018, Kerala, India

*Shellfish Fisheries Division, ICAR-Central Marine Fisheries Research Institute, Ernakulam North P. O., P.B. No. 1603, Kochi - 682 018, Kerala, India

e-mail: kajal_cmfri@yahoo.com; kajal.chakraborty@icar.gov.in

ABSTRACT

Seasonal variations of the fatty acid profile of deep-sea whip lobster *Puerulus sewelli* (Decapoda, Palinuridae), harvested from the south-west coast of India were compared. Fatty acid contents showed seasonal fluctuations, with eicosapentaenoic acid and docosahexaenoic acid being predominant during the post-monsoon and winter seasons (9-11% of cumulative fatty acids). *P. sewelli* was found to possess considerably higher C_{20-22} *n*-3 fatty acids during these seasons along with balanced *n*-3/*n*-6 (>2) and polyunsaturated/saturated fatty acid ratio (≥ 1) than those in pre-monsoon. Greater hypocholesterolemic/hypercholesterolemic ratio (>2.4) and lesser atherogenicity (<1.8) and thrombogenicity (<0.3) indices qualify *P. sewelli* an ideal health food. This study comprises the first report on fatty acid profile from commercially important deep-sea whip lobster *P. sewelli*.

Keywords: Atherogenicity index, Non-conventional resource, Polyunsaturated fatty acid, Seafood benefits, Thrombogenicity index

Deep-sea lobsters are recognised as non-conventional culinary delicacies owing to the occurrence of high-quality long-chain highly polyunsaturated fatty acids (PUFAs). However, they continued to persist as one of the predominantly unexplored marine fishery resources. Spiny lobster *Puerulus sewelli* (Decapoda, Palinuridae) is one of the most prominent deep-sea lobster species distributed in the coastal part of peninsular India. It is noteworthy to mention that this species is one of the most valuable deep-sea crustaceans globally and has earned foreign exchange in both live and frozen forms because of the presence of high contents of PUFAs, specifically *n*-3 PUFAs, for example docosahexaenoic acid (DHA, 22:6*n*-3) and eicosapentaenoic acid (EPA, 20:5*n*-3) that shows defensive strategies aiding human health in many ways. The *n*-3 PUFAs were recognised to biosynthesise anti-inflammatory resolvins other than pluralities of bioactivities, such as antithrombotic effects, in addition to impart important role to control the pathophysiology causing the state of hypertension, diabetes and cancer (Simopoulos, 2001; Schmidt, 2003; Sidhu, 2003; Mahaffey, 2004). Furthermore, it is also considered as an important culinary delicacy worldwide and could be incorporated into a healthy diet as it contains low saturated fat and high protein content (FNB, 2007).

The marine crustacean species were reported to possess a considerable amount of *n*-3 PUFA (Berge and

Barnathan, 2005), which along with the EPA and DHA plays a prominent role in the growth and maintenance of the body. Inflammatory eicosanoids could find their origin in *n*-6 PUFA and are inflammatory, whereas those resulting from *n*-3 PUFAs were bestowed with potential anti-inflammatory activities (Calder, 2004). Consequently, health organisations have advocated a cumulative EPA and DHA intake of about 250-500 mg daily for healthy adults (Hjalmarsdottir, 2019).

Studies on nutritional profile in terms of the fatty acid composition of seafood based on seasonal variation have received close attention and considering *P. sewelli* as a candidate species would be ideal. It was documented that the seasonal alterations could regulate the fatty acid profile in marine crustaceans (Yanar and Celik, 2005). Differences in sea temperature are an imperious element, which can affect fatty acid unsaturation in these species. This work anticipated to assess the comparative fatty acid profile in the edible part of deep-sea whip lobster *P. sewelli* Ramadan, 1938 (family Palinuridae) in three different seasons namely, post-monsoon (October and November); winter (December-February) and pre-monsoon or summer (March-May). As seasonal variations could shape the fatty acid profile, the logic of this study was to institute the effects of season on the fatty acid composition of the edible parts of *P. sewelli*. This could also provide

us with valuable information regarding the availability or peak catch period *vis-a-vis* fatty acid composition of the edible parts of this deep-sea lobster species.

Fresh *P. sewelli* was collected from the Kollam coast (8°56' N; 76°32' E) along south-west peninsular India (Fig. 1a). Three independent samples were collected during the post-monsoon, winter and pre-monsoon seasons. The collected samples were then transferred to the laboratory in ice box and were stored at -20°C for further studies. The sample was processed by removing the outer shell and the edible tissue was minced for further analyses and values were expressed as the mean of triplicates.

To analyse the fatty acid composition of the edible part of *P. sewelli* (Chakraborty *et al.*, 2014), chloroform:methanol solvent mixture (1:2, v v-1) was used to extract the lipid fraction, which was saponified before being *trans*-esterified to yield fatty acid methyl esters (FAMES). The latter was extracted with *n*-hexane:water solvent mixture (1:2, v v-1) and the *n*-hexane layer was passed through anhydrous sodium sulfate. A rotary vacuum evaporator concentrated the clarified solvent layer to yield esterified fatty acids, which were fractionated by a capillary column (SP®2560; 100 m × 0.25 mm and 0.50 µm film thickness, Supelco, USA) mounted on a GLC (Gas Liquid Chromatograph, AutoSystemXL Perkin-Elmer, USA) apparatus. A flame ionisation detector (FID) detected the fractionated esterified fatty acids. GLC conditions were detailed in earlier literature (Chakraborty *et al.*, 2014). Esterified fatty acids were quantified by comparing the retention times of known fatty acid standards (Supelco™ 37-FAME-mix) and the results were expressed as per cent total/cumulative fatty acids (% CFA).

Fatty acid ratios [PUFA/saturated fatty acid (SFA), DHA/EPA and *n*-3/*n*-6] representing nutritional qualities of *P. sewelli* were calculated (HMSO, 2001) in addition to thrombogenic and atherogenic indices (TI and AI, respectively) using monounsaturated fatty acid (MUFA), and PUFA (Ulbricht and Southgate, 1991):

$$TI = (14:0 + 16:0 + 18:0) / [(0.5 \times \sum MUFA) + (0.5 \times \sum n-6 PUFA + (3 \times \sum n-3 PUFA) + (\sum n-3 PUFA / \sum n-6 PUFA))];$$

$$AI = (4 \times 14:0 + 18:0 + 16:0) / (\sum MUFA + \sum n-3 PUFA + \sum n-6 PUFA).$$

The hypocholesterolaemic/hypercholesterolaemic (h/H) ratio was quantified (Santos-Silva *et al.*, 2002):

$$HH = (C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C14:0 + C 16:0).$$

Statistical Program for Social Sciences (SPSS ver. 13.0, USA) was used to calculate significant differences among the means ($p < 0.05$) by one-way analysis of variance (ANOVA). The significant differences in fatty

acid content (as % CFA) indicated by ANOVA were further tested using Scheffe's Post Hoc Test. A principal components analysis (PCA) was used to represent the similarities and dissimilarities of the seasons of the various fatty acids studied.

Oceanic lobsters are considered among the most delicious seafood items. In addition to their delicacy, they are recognised as potential sources of long-chain C20-22 *n*-3 PUFAs, which have significant biomedical implications (Ramezani-Fard *et al.*, 2016). Fatty acids are valuable sources of essential nutrients and carriers of lipid-soluble vitamins and were reported to retain cell membrane networks (Bhavan *et al.*, 2010). The GC profile of FAMES derived from the lipid fraction of *P. sewelli* is depicted in Fig. 1b.

Seasonal differences in fatty acid constituents in *P. sewelli* are illustrated in Table 1. The most abundant SFA was palmitic acid (16:0), comprising about 10-12% CFA. The mean total content of 16:0 was considerably lower ($p > 0.05$) during the winter (10.7% CFA) and peaked during the pre/post-monsoon months (~12%). The principal source of energy for crustaceans and marine fishes is SFAs, principally 16:0, as a result of substantially higher calorific values (Henderson *et al.*, 1984; Chandrani *et al.*, 2012). The concentration of SFAs could increase during more significant feeding activity during the pre-monsoon season (Shirai *et al.*, 2002). On the contrary, the per cent share of 16:0 was lesser during the months between December and February, thereby recognising that these storage fatty acids might degrade to supply energy to the crustacean owing to the more secondary feeding activities during this period. Stearic acid (18:0) was found to be the next most rich SFA, and no significant differences in its content (~9% CFA) in the samples collected during different seasons were apparent. The level of odd-chain saturated fatty acids (15:0) presented a significant quantity (~5%) during various seasons.

The MUFAs exhibited a decreased presence (<24% CFA) in the samples of *P. sewelli* collected during the winter months (December-February) and after that, an increase (29% CFA) during pre-monsoon season was perceived. Palmitoleic (16:1*n*-7) and oleic (18:1*n*-9) acids were found to be the main fatty acids in both species and sexes. Considerably higher 18:1*n*-9 content was perceived in the edible part of *P. sewelli* (12-13% CFA) collected during the pre-monsoon and post-monsoon months. Likewise, 16:1*n*-7 content was significantly higher in the summer (March and May) and post-monsoon seasons (October and November), in the studied species ($p < 0.05$) than that recorded in the edible part of *P. sewelli* collected during the colder months (December-February) (~3% CFA). Oleic acid was recognised for energy metabolism (Huynh

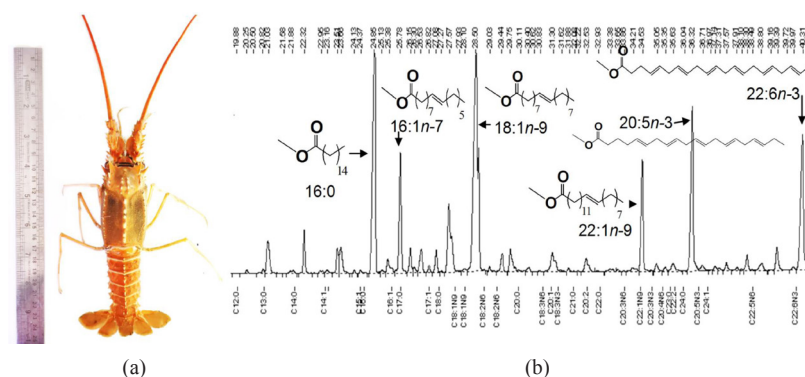


Fig. 1. (a) Representative photograph of the deep-sea whip lobster *P. sewelli* collected by the commercial deep-sea trawlers operated off Quilon (Kerala State), along the south-east coast of the peninsular India; (b) Gas chromatographic profile of FAMES derived from the lipidic extract of the edible portion of *P. sewelli*. FAMES were identified by comparison of retention time (R_p , min) with known standards. Results are expressed as percent of cumulative fatty acids (% CFA)

et al., 2007) and was the major MUFA in crustaceans during pre-monsoon. These results were consistent with the previous accounts that the principal MUFAs in marine lipids characteristically comprise eighteen carbon frameworks (Zlatanov and Laskaridis, 2007). The quantities of 18:1*n*-9 in the deep-water crustaceans were more significant than 16:1*n*-7, which aligned with previous outcomes (Ouraji *et al.*, 2009).

Polyunsaturated fatty acids (PUFAs) were found to be the predominant fatty acids in the total lipid content of *P. sewelli*. PUFA was found to contain a considerably higher share of *n*-3 fatty acids, notwithstanding the seasons, thereby making the fatty acid profile of this species a source of healthy food. The PUFA of *P. sewelli* collected during the post-monsoon season and winter months exhibited significantly higher values (up to 37% CFA) than that recorded during pre-monsoon (29% CFA) ($p < 0.05$). The *n*-3 PUFAs identified in the edible part of *P. sewelli* were DHA and EPA. Yanar and Celik (2005) described that these long-chain polyenoic acids are critical fatty acids in the lipids of marine crustaceans. There was evidence that EPA and DHA could decrease the risk of cardiovascular disease and reduce serum cholesterol (Weichselbaum *et al.*, 2013). Similarly, the *n*-6 series of PUFAs were arachidonic acid (20:4*n*-6), linoleic acid (18:2*n*-6) and γ -linolenic acid (18:3*n*-6). The total *n*-3 PUFAs were considerably higher in the samples collected during October-February (~23% CFA) than those collected during the summer months (18% CFA). The content of EPA was higher during the post-monsoon months (10% CFA), where the C22 polyenoic fatty acid (DHA) exhibited a winter maximum (11% CFA) in the edible part of *P. sewelli*. DHA was found to possess higher biological value than EPA (Swanson *et al.*, 2012) and can be reserved more effectively than EPA by marine fish, most likely as a result of the intrinsic complexities to oxidise

this fatty acid that necessitates the peroxisomal conduit of α -oxidation for EPA (Sargent *et al.*, 2002). Moreover, marine species with higher DHA necessity could select to accrue more DHA than EPA in the body (Swanson *et al.*, 2012). The total PUFA was significantly higher in the colder months and through the post-monsoon and winter months (> 20% CFA), essentially, a higher degree of fatty acid unsaturation in the studied species was perceived. The content of EPA+DHA was maximum during the winter season, apparently owing to the relatively lesser temperature resulting in the up-regulation in the desaturase and elongase to biosynthesise larger contents of C20-22 *n*-3 PUFAs. This has been confirmed by the higher share of long-chain PUFAs in marine crustaceans during winter (Soriguer *et al.*, 1997). An increased percentage of long-chain *n*-3 PUFAs during the colder months might increase the membrane fluidity to facilitate marine organisms thriving at lower environmental temperatures. Additionally, lesser temperature might increase the activities of elongase and desaturase (Craig *et al.*, 1995). Linoleic acid was the most critical *n*-6 PUFA in *P. sewelli* during the studied seasons (2-3% CFA). Arachidonic acid was one of the major *n*-6 PUFAs in *P. sewelli* and could be characteristically related with tissue membrane and is the key component of cells (Ramezani-Fard *et al.*, 2016). The information on seasonal deviations in the composition of fatty acid of *P. sewelli* would also assist in identifying the proper lobster harvesting period, which can maximise nutritional benefits from the species. Further, an appropriate perception of the biochemical elements of *P. sewelli* has become a key necessity for dietitians along with the utilisation for nutritional and lobster processing industries.

The *n*-3/*n*-6 ratio, a marker of biomedical connotation, accounted for 2-2.5 in *P. sewelli* throughout different seasons. Conspicuously, the peak catch period of the

Table 1. Seasonal variability of fatty acid composition of the edible tissues of *P. sewelli*

Fatty acids	Summer [‡]	Post-monsoon [§]	Winter [¶]
Saturated fatty acids			
12:0	1.43 ^b ±0.04	0.55 ^a ±0.03	2.62 ^c ±0.01
14:0	2.16 ^a ±0.02	1.74 ^a ±0.03	5.01 ^b ±0.01
15:0	5.47 ^b ±0.21	4.55 ^a ±0.03	5.25 ^b ±0.05
16:0	12.27 ^b ±0.02	12.36 ^b ±0.05	10.73 ^a ±0.03
17:0	2.10 ^b ±0.02	0.36 ^a ±0.08	1.56 ^b ±0.10
18:0	8.80 ^a ±0.06	8.81 ^a ±0.01	9.04 ^a ±0.05
20:0	0.75 ^c ±0.04	0.67 ^b ±0.41	0.56 ^a ±0.03
22:0	0.51 ^b ±0.03	0.63 ^c ±0.03	0.45 ^a ±0.02
24:0	0.58 ^a ±0.06	0.51 ^a ±0.02	1.46 ^b ±0.03
ΣSFA [†]	34.3 ^b ±0.28	30.46 ^a ±0.16	36.73 ^c ±0.08
Monounsaturated fatty acids			
14:1 n -7	1.06 ^c ±0.06	0.65 ^a ±0.04	0.84 ^b ±0.06
15:1	0.53 ^b ±0.04	0.25 ^a ±0.02	0.24 ^a ±0.02
16:1 n -7	4.16 ^b ±0.04	4.32 ^b ±0.01	3.42 ^a ±0.03
18:1 n -7	0.93 ^b ±0.12	0.55 ^a ±0.15	0.46 ^a ±0.08
18:1 n -9	12.19 ^b ±0.14	13.01 ^c ±0.18	11.35 ^a ±0.22
20:1	1.58 ^b ±0.14	2.18 ^c ±0.26	1.00 ^a ±0.08
22:1	6.12 ^b ±0.16	6.51 ^c ±0.32	5.92 ^a ±0.26
24:1	2.12 ^c ±0.34	1.47 ^b ±0.19	0.94 ^a ±0.06
ΣMUFA [§]	28.61 ^b ±0.26	29.02 ^b ±0.33	24.12 ^a ±0.12
Polyunsaturated fatty acids			
16:2 n -4	0.40 ^b ±0.06	0.54 ^c ±0.04	0.31 ^a ±0.04
16:3 n -4	0.25 ^a ±0.02	0.38 ^b ±0.02	0.29 ^a ±0.05
18:2 n -6	2.70 ^a ±0.05	3.13 ^b ±0.04	2.51 ^a ±0.38
18:3 n -6	1.33 ^a ±0.12	3.48 ^b ±0.16	1.95 ^a ±0.18
18:3 n -3	0.37 ^a ±0.13	0.51 ^b ±0.19	0.29 ^a ±0.06
20:2 n -6	2.45 ^a ±0.13	2.35 ^a ±0.25	2.84 ^b ±0.18
20:3 n -6	1.27 ^b ±0.11	0.57 ^a ±0.04	0.70 ^a ±0.08
20:4 n -6 (AA)	1.84 ^a ±0.12	2.26 ^b ±0.21	1.90 ^a ±0.09
20:5 n -3 (EPA)	8.33 ^a ±0.32	10.02 ^c ±0.26	9.47 ^b ±0.58
22:5 n -3	2.02 ^a ±0.24	2.63 ^c ±0.12	2.44 ^b ±0.16
22:6 n -3 (DHA)	7.80 ^a ±0.31	10.71 ^b ±0.46	11.2 ^c ±0.62
ΣPUFA [¶]	28.65 ^a ±0.16	36.59 ^c ±0.21	33.6 ^b ±0.28
Fatty acid-based nutritional indices			
Σ n -3 PUFA	18.52 ^a ±0.13	23.9 ^b ±0.29	23.38 ^b ±0.14
Σ n -6 PUFA	9.57 ^a ±0.08	11.73 ^c ±0.32	9.66 ^b ±0.21
Σ n -3/Σ n -6 PUFA	1.96 ^a ±0.08	2.03 ^a ±0.14	2.49 ^b ±0.22
18:1 n -7/ n -9	0.07 ^a ±0.02	0.05 ^a ±0.01	0.04 ^a ±0.01
DHA + EPA	16.18 ^a ±0.15	20.75 ^b ±0.12	20.68 ^b ±0.18
EPA/DHA	1.09 ^b ±0.06	0.95 ^a ±0.16	0.85 ^a ±0.16
ΣPUFA/ΣSFA	0.85 ^a ±0.04	1.24 ^b ±0.12	0.96 ^a ±0.08
AI	1.66 ^b ±0.06	1.44 ^a ±0.04	1.75 ^b ±0.09
TI	0.29 ^a ±0.04	0.23 ^a ±0.12	0.25 ^a ±0.08
HH	2.45 ^a ±0.13	3.28 ^b ±0.17	2.48 ^a ±0.12

[†]ΣSFA: Total saturated fatty acids

[§]ΣMUFA: Total monounsaturated fatty acids

[¶]ΣPUFA: Total polyunsaturated fatty acids.

Individual fatty acids were expressed as percentage of total identifiable fatty acids. Data are presented as mean values of three samples (mean±standard deviation). Means bearing different superscripts (a-c) within the same row indicate significant differences ($p < 0.05$).

species is during the months of December-February, which coincides with the considerably higher n -3/ n -6 ratio during the winter season (2.5; Table 1) than those in post-

monsoon and summer months (~2). Earlier studies have demonstrated that *P. sewelli* is abundantly available in the fishing grounds during January-April and sporadically

distributed afterwards. It is important to note that the principal recruitment to this population occurs during October. In contrast, during January-February, this lobster species is found to be in greater abundance (265-522 kg h⁻¹) in the depth zone of 151-250 m, and it gives the idea that with the appearance of the summer season, it migrates to deeper waters (Kathirvel *et al.*, 1989). Studies suggest that a greater level of dietary *n*-6 PUFA could result in quite a few health disorders, even as *n*-3 PUFAs alter the negative effects of *n*-6 PUFA (Calder, 2004). The *n*-3/*n*-6 ratio is significant for optimum synthesis of eicosanoids (Steffens, 1997) and long chain *n*-3 PUFAs, particularly those belonging to C20-22 analogous could decelerate the overproduction of inflammatory eicosanoids. Consistent with the UK Department of Health, an *n*-6/*n*-3 ratio within 0.2-1.5 would set up a portion of healthy food. In contrast, a greater *n*-6/*n*-3 (> 1.5) would be risky and may eventually lead to cardiovascular ailments (Wijendran and Hayes, 2004). The *n*-3/*n*-6 ratio of the edible part of *P. sewelli* was contained by the recommended range and subsequently had promising significance as a functional food. This study also showed that the edible part of *P. sewelli* has a superior PUFA/SFA ratio than the recommended least value of 0.45 (HMSO, 2001), during the studied seasons. Conspicuously, a greater level of PUFA to SFA (more than 0.45) in diet may possibly reduce cardiovascular diseases. The AI and TI of

P. sewelli were 1.4-1.7 and 0.2-0.3, respectively (Table 1); therefore, this deep-water lobster could be a candidate for healthy seafood. No noteworthy difference in the HH ratio among different seasons ($p > 0.05$) was perceived. AI indicates the possibility of cardiovascular diseases, while TI is sign of a conglomeration of blood platelets. The *n*-3 PUFAs are essential to attenuate atherosclerosis and aggregation of platelets and exhibit potent antiatherogenic and antithromobogenic activities (Barrento *et al.*, 2010).

PCA represented the similarities and differences between the seasons of fatty acids. The parameters assessed were Σ SFA, Σ MUFA, Σ PUFA, Σ *n*-3 PUFA, Σ *n*-6 PUFA, EPA and DHA. The PC1 and PC2, the first and the second order principal variance, represented 56.21 and 43.78%, respectively. PC1 was fundamentally influenced by Σ *n*-3 PUFA and Σ MUFA of post-monsoon and summer seasons, respectively. PC2 was impacted by Σ *n*-6, EPA and Σ PUFA of the winter season (Fig. 2a). Furthermore, a strong positive correlation was perceived between Σ PUFA and EPA ($R^2 = 0.986$) (Fig. 2b) and Σ *n*-3 and DHA+EPA ($R^2 = 0.995$) (Fig. 2c).

Consumption of deep-sea lobsters has been increasing worldwide during the previous decades owing to their potential nutritional values that depict a substitute for the over-exploited fishery resources. The present

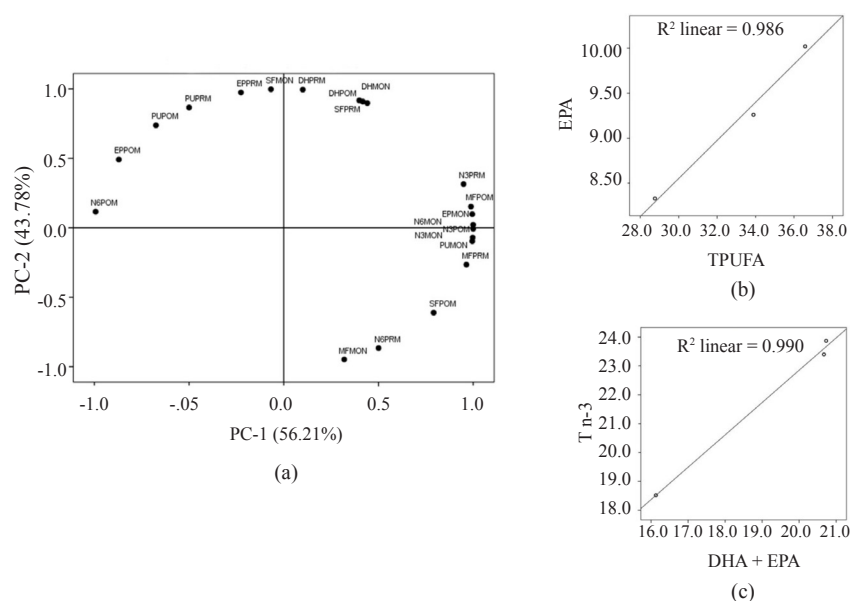


Fig. 2. (a) Loading plot diagram representing the correlation of total *n*-3 fatty acids, EPA, DHA and fatty acid indices of *P. sewelli* during pre-monsoon, monsoon and post-monsoon months. N6POM: *n*-6 post-monsoon, N6PRM: *n*-6 pre-monsoon, N6MON: *n*-6 monsoon, N3POM: *n*-3 post-monsoon, N3PRM: *n*-3 pre-monsoon, N3MON: *n*-3 monsoon, EPPOM: EPA post-monsoon, EPPRM: EPA pre-monsoon, EPMON: EPA monsoon, DHPOM: DHA post-monsoon, DHPRM: DHA pre-monsoon, DHMON: DHA monsoon, SFPOM: SFA post-monsoon, SFPRM: SFA pre-monsoon, SFMON: SFA monsoon, PFPOM: PUFA post-monsoon, PFPRM: PUFA pre-monsoon, PFMON: PUFA monsoon. (b) Correlation plot between total PUFA (TPUFA) and EPA content of *P. sewelli* during pre-monsoon, monsoon and post-monsoon months. (c) Correlation plot between total EPA+DHA and *n*-3 (T_{n-3}) content of *P. sewelli* during pre-monsoon, monsoon and post-monsoon months

study established the fatty acid compositions important for their inclusion as high-health dietary substances. A comprehensive analysis of the fatty acid composition of *P. sewelli* during various seasons revealed that those harvested during the post-monsoon and winter seasons, were nutritionally superior concerning C20-22 n-3 PUFs contents and other nutritional quality indices. Lesser atherogenic and thrombogenicity indices could conform to the ideal dietetic characteristics of the edible part of *P. sewelli*, which are of enormous commercial implications, and feature balanced nutritional qualities as a healthy food.

Acknowledgements

This work was supported by the Indian Council of Agricultural Research (ICAR), New Delhi, under the Fisheries Science Division Outreach Activity on Nutrient Profiling and Evaluation of Fish as a Dietary Component [Grant No.ICAR-FSD-OA#3]. The authors thank the Director, ICAR-CMFRI, Kochi, for guidance and support. S.K. acknowledges the ICAR, for the award of a scholarship. Thanks are due to the Heads, Marine Biotechnology, Fish Nutrition and Health Division and Shellfish Fisheries Division of ICAR-CMFRI, Kochi, for support.

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