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Deciphering the Stock Structure of White Sardine *Escualosa thoracata* (Valenciennes, 1847) along the Indian Waters by using Chemometric Analysis of Natural Signature Fatty Acid Profile

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ABSTRACT

Background: Small pelagic species like white sardine gained importance due to their high demand in local markets over years has become important to sustainably harvest the available resources. Thus, the current study was aimed to decipher the stock structure studies based on chemometric analysis of natural signature fatty acid profile which plays an important role in stock identification to the formulation of better sustainable management plans and its conservation.

Methods: Samples of *Escualosa thoracata* were collected from four locations each from two on the east coast (Chennai and Kolkata) and west coast (Mumbai and Kochi) of the Indian peninsula 2019-2020. Heart tissue from the specimen from each location was collected, preserved and fatty acid profiling was performed. The analysis comprised methanolysis, gas chromatography and multivariate statistics.

Result: The profile showed a difference between southern and northern populations on both the coast. The trend was more obvious in SAFAs and PUFAs; SAFAs were high content in the southern populations and PUFAs were high content in the northern populations. The separation can be attributed to the latitudinal effects on the deposition of fatty acids.

Key words: Chemometric, *Escualosa thoracata*, Fatty acid, Stock structure.

INTRODUCTION

The lipids play important roles in the life histories and physiology of fish. Lipids and their constituent fatty acids, along with protein, are the major organic constituent of fish. Fatty acids (FA), as one of the most important components of lipids, are associated with growth and development. The differences in the proximate composition of fish are associated with their feed intake, variations in the lipid content are much larger than protein and minerals (Bagthasingh *et al*., 2016). Lipid plays source of metabolic energy in fish for reproduction and movement, including migration. Moreover, fish lipids' fatty acids are rich in long-chain, highly unsaturated fatty acids (HUFA) that have particularly important roles in human nutrition. When fatty acid profiles are used for the identification of fish stocks, the assumption is that the composition of fatty acids in membrane phospholipids is genetically controlled and stable over time.

More recent studies suggested that the percentage composition of phospholipid fatty acids prominent in some body tissues (heart tissue, brain, eggs) have a genetic basis that analyses these tissues appropriate for stock structure studies (Joensen and Grahl-Nielsen, 2000; Joensen *et al*., 2000). Fatty acids are considered as a critical tool to be analysed for biochemical and physiological standards of a known organism (Shahina *et al*., 2016; Ahmed *et al*., 2017). A chemometric method for classification of samples based on fatty acid composition has been developed at the department of chemistry, the University of Bergen, which may be used for stock identification (Grahl-Nielsen and Mjaavatten, 1992; Grahl-Nielsen *et al*., 1993) and has been included in the listing of ICES stock identification methodologies (Grahl-Nielsen, 2005).

Thus, the present study based on chemometric analysis of fatty acid profile was undertaken to delineate the stock of geographically distant population of *Escualosa thoracata* in the west coast and east coast which will be helpful to formulate the sustainable management strategies.

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MATERIALS AND METHODS

Fatty acid profile-based analysis

For this study, samples of *E. thoracata* were collected from the above four locations in the year 2019-2020. The heart is dissected out and blood is carefully removed by washing in normal saline. The tissue was dried on filter paper and transferred into thick-walled glass tubes with Teflon-lined screw caps containing Chloroform-Methanol (2:1) solution. Extraction of lipids was done according to the procedure of Folch *et al*., (1957). The heart tissue was macerated well with the mixture of chloroform and methanol. It was filtered into a separating funnel added with 1/5th volume of NaCl solution and kept overnight for separation. The lower lipids layer was collected into a pre-weighed round bottom flask and evaporated to dryness under vacuum using an evaporator. The final weight of the flask was taken to calculate the weight of actual lipid. The AOAC (1995) method was used for esterification of the lipid extract. FAME was prepared from the lipids extracted from the fish heart sample by heating with the methanolic NaOH first and then with BF3 Methanol for esterification. FAMEs were extracted in hexane and for this purpose, 5 ml n-heptane was added to recover the methyl esters in organic were separated using a separating funnel. The upper n-heptane phase was taken out then stored in 10 ml glass vials in the refrigerator until further analysis.

Gas Chromatography-Mass Spectrometry

Fatty acids were separated by using a Shimadzu QP2010 quadrupole Gas Chromatography-Mass Spectrometer (GC-MS) instrument equipped with a Carbowax (30m* 0.25 mm Id; 0.25- um film thickness) capillary column (Cromlab S.A.) helium gas was utilized as a carrier the temperature at 250°C was set as Injector and detector. Split mode (1:15) was used in the injection. The column temperature was programmed initially at 50°C for 2 mins and then to increase at a rate of 10°C per min to a final temperature of 230°C. At 23.1 kPa (constant pressure) the fatty acid methyl esters were separated.

Data analysis

PROC MEANS procedure (SAS Institute, 2000) was used to estimate the descriptive statistics for fatty acid profile analysis. Principal component analysis (PCA): The relative value of the fatty acids was transformed to level out the difference among fatty acids present in large and small amounts and incorporate samples with missing values for any of the fatty acids by using the following formula.

$$X = \log (X+1)$$

Where,
X = Transformed variable.
X= Original variable.

The transformed data were used to Principal Component Analysis (PCA) using PROC PRINCOMP procedure of SAS (Hatcher, 2003).

RESULTS AND DISCUSSION

The relative area percentage was obtained for 25 fatty acids, comprising six mono unsaturated fatty acids (MUFA): 16:In-7, 16:In-5, 18:In-9, 18:In-7, 18:In-5 and 20:In-9 and eleven poly unsaturated fatty acids (PUFA): 16:2n-6, 18:2n-6, 18:3n-3, 20:2n-7, 20:3n-7, 20:4n-6, 20:4n-3, 20:5n-3, 22:5n-6, 22: 5n-3 and 22: 6n-3. Eight saturated fatty acids (SAFA): 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 19:0 and 20:0. The estimated mean and standard error for the fatty acid profile data from four locations *i.e*. Kolkata, Chennai, Cochin and Mumbai populations of *E. thoracata* are given in Table 1.

Saturated fatty acids (SAFA)

The most common saturated fatty acids are palmitic acids (PA C16:0), lauric acid (LA C12:0), myristic acid (MA C14:0) and stearic acid (SA C18:0) (Sekar *et al.,* 2017). All the eight saturated fatty acids (SAFA) were recorded from Chennai and Cochin populations, whereas in the sample of the Kolkata population, seven of them, except lauric acid (12:0), were recorded. Seven saturated fatty acids, except eicosanoic acid (20:0) also contained in Samples of the Mumbai location. The mean value of all of the eight saturated fatty acids was the lowest in the samples in the Kolkata population followed by the Mumbai population. In Chennai, five of the eight SAFA, *i.e*. 12:0, 14:0, 15:0, 16:0 and 18:0, were highest among the four locations and the rest of three SAFA, *i.e*. 17:0, 19:0 and 20:0 were highest percentage composition in Cochin samples. The highest percentage composition showed by Palmitic acid (16:0) in the samples of all the locations followed by Stearic acid (18:0) ranged from 14.94% in Kolkata samples to 27.12% in Chennai samples. All of the eight SAFAs showed a significant difference in their least-square means among any of the four sampling locations. None of the SAFA showed a significant difference between Kolkata and Mumbai populations, whereas six of the eight SAFA (except 17:0 and 19:0), showed a significant variation between Chennai and Mumbai locations. Five SAFA (14:0, 15:0, 16:0 and 18:0) showed significant variation between Kolkata and Chennai populations and four SAFA, (15:0, 16:0, 19:0 and 20:0) between Chennai and Cochin populations.

Monounsaturated fatty acids (MUFA)

Three MUFA *i.e*. palmitoleic acid (16:In-7), oleic acid (18:In-9) and gondoic acid (20:In-9) were obtained from all the four sampling locations. Two MUFA *i.e*. vaccine acid or asclepic acid (18:In-7) and 11-cis hexadecenoic acid (16:In-5) were obtained from Kolkata and Cochin samples whereas 18:In-5 was obtained from Cochin and Mumbai. Among MUFA, the highest percentage composition showed by oleic acid followed by palmitoleic acid and gondoic acid. Among palmitoleic acid, oleic acid and gondoic acid, only oleic acid showed a significant difference between locations and it was highest in Mumbai samples, followed by Cochin. The vaccinic or asclepic acid showed significant variation among Kolkata and Cochin samples.

Poly unsaturated fatty acids (PUFA)

Fish is known to be a rich source of omega-3 poly unsaturated fatty acid especially eicosapentaenoic (EPA)

Table 1: Relative amounts as percentage of Sum±SE of fatty acids in heart tissue of *E. thoracata*.

Fatty acid	% Fatty acids Mean±S.E.			
	Kolkata	Chennai	Cochin	Mumbai
SAFA				
12:0	-	1.55^{a}±0.27	1.02^{a}±0.19	0.56^{b}±0.18
14:0	1.02^{a}±0.23	3.45^{b}±0.45	2.65^{b}± 0.13	1.56^{a}±0.58
15:0	0.41^{a}±0.10	0.68^{b}± 0.09	0.66^{a}±0.04	0.23^{a}±0.05
16:0	27.45^{a}±1.65	36.55^{b}±0.10	35.11^{a}±1.07	29.82^{a}±2.45
17:0	1.01^{a}±0.19	1.57^{ab}±0.04	1.65^{b}±0.06	1.60$^{a\ b}$± 0.12
18:0	14.94^{a}±0.82	27.12^{b}±1.23	25.74^{b}±0.56	19.33^{a}±1.22
19:0	0.31^{a}±0.10	0.36^{a}±0.02	0.77^{b}±0.08	0.47$^{a\pm}$0.11
20:0	0.13^{a}±0.02	0.23^{a}±0.07	0.64^{b}± 0.05	-
MUFA				
16:1n-7	0.89^{a}±0.22	0.85^{a}±0.21	1.69$^{a\ b}$± 0.17	2.16^{b}± 0.35
16:1n-5	0.14^{a}±0.03	-	0.14^{a}±0.04	-
18:1n-9	7.85^{a}±1.17	7.13^{a}±1.20	7.38^{a}±0.28	8.58^{a}±1.10
18:1n-7	0.25^{a}±0.20	-	1.97^{b}±0.73	-
18:1n-5	-	-	1.59^{a}±0.65	2.75^{b}±0.32
20:1n-9	0.15^{a}±0.03	0.20^{a}±0.03	0.26^{a}±0.15	0.12^{a}±0.05
PUFA				
16:2n-6	0.41^{a}±0.23	-	-	0.21^{a}±0.15
18:2n-6	1.33^{a}±0.28	1.09^{a}±0.25	1.18^{a}±0.32	1.59^{b}± 0.24
18:3n-3	-	-	-	0.22± 0.07
20:2n-7	0.06^{a}±0.05	-	0.44^{b}± 0.05	0.07^{a}±0.06
20:3n-7	0.34^{a}±0.03	-	0.04^{b}± 0.05	-
20:4n-6	4.37^{a}±0.44	2.56^{a}±0.82	3.56^{a}±0.25	3.08^{a}±0.35
20:4n-3	0.83^{a}±0.15	0.22^{b}±0.17	0.16^{b}±0.04	0.76^{a}±0.33
20:5n-3	1.89^{a}±0.65	-	1.47^{b}±0.33	2.36^{a}±0.22
22:5n-6	1.56^{a}±0.33	1.34^{a}±0.32	0.79^{b}± 0.43	0.87^{b}±0.25
22:5n-3	1.54^{a}±0.38	-	0.54^{b}±0.48	1.75^{a}±0.22
22:6n-3	25.45^{a}±0.73	16.07^{b}±0.83	20.15^{b}±0.89	24.02^{a}±2.09

and docosahexaenoic acid (DHA) (Cascant *et al.,* 2018). Total of eleven PUFA was obtained from all the samples of all four locations in which five, (linoleic acid (18:2n-6), arachidonic acid (20:4n-6), eicosatetrainoic acid (ETA;20:4n-3), osbond acid (22:5n-6) and docosahexaenoic acid (DHA;22:6n-3) were found in samples of all the four locations. Alpha-linolenic acid (ALA:18:3n-3) was found in the samples of Mumbai only. The fatty acids, 20:2n-7, eicosapentaenoic acid (EPA; 20:5n-3) and decosapentaenoic acid (DPA; cluspandonic acid; 22:5n-3) were present in three populations Kolkata, Cochin and Mumbai. The highest percentage in DHA showed in all populations followed by Arachidonic acid in populations of all locations. Eicosatetraenoic acid and DHA were found to be in significantly high concentrations in Kolkata and Mumbai populations as compared to Chennai and Cochin. Mumbai population contained a significantly high concentration of Linoleic acid compared to other the three locations. No significant variation in Arachidonic acid was observed among the four populations Kolkata, Cochin, Mumbai and Chennai. Osbond acid showed a significantly high percentage composition in the east coast populations as compared to west coast populations.

Principal component analysis (PCA)

PCA analysis of the fatty acid profile data of four sampling locations *i.e*. Kolkata, Chennai, Cochin and Mumbai revealed the fatty acids which are liable to change according to changes in locations. Total variation together explained 72.45 per cent in the first two principal components (PCs) with eigenvalues 132.23 and 6.11 respectively. PC1 depicted 77.30 percent of total variation whereas PC2 accounted 4.12 percent of the same (Table 2). The fatty acids with meaningful loading on PCI were lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0) and stearic acid (18:0). The maximum loading on PC1 was by palmitic acid followed by stearic acid. All the n-3 and n-6 fatty acids resembled negative loadings on PC1. DHA (22:6n-3) showed the highest negative loading on PC1 followed by ETA (20:4n-3) (Table 3). The second component, PC2 which explained 4.12 percent of total variation were loaded with fatty acids ALA (18:3n-3), ETA (20:4n-3), EPA (20:5n-3), DPA (22:5N-3) and

Table 2: Eigen values and proportions of variance contribution to the total variance of fatty acid profile data in the *Escualosa thoracata* from the four locations.

Component	Eigen value	Difference	Proportion %	Cumulative %
PC1	132.23	126.12	77.30	77.30
PC2	6.11	-	4.12	81.42

Table 3: Variable loadings in Principal Component Analysis of fatty acid profile data.

Fatty acid	PC1	PC2
SAFA		
12:0	0.2078	-0.2342
14:0	0.3567	-0.3967
15:0	0.0067	-0.4123
16:0	0.6022	-0.4987
17:0	0.0145	-0.1211
18:0	0.4033	-0.3323
19:0	0.0005	0.0089
20:0	0.0044	-0.0044
MUFA		
16: 1n-7	0.0033	0.1543
16:1n-5	-0.0211	-0.2230
18:1n-9	-0.0006	0.1567
18:1n-7	0.0223	-0.2127
18:1n-6	-0.1156	0.1477
20:1n-9	0.0234	-0.0121
PUFA		
16:2n-6	-0.0287	-0.0012
18:2n-6	-0.3134	0.1434
18:3n-3	-0.0346	0.2335
20:2n-7	0.1566	-0.0045
20:3n-7	-0.1456	-0.2033
20:4n-6	-0.0455	-0.0654
20:4n-3	-0.3223	0.2068
20:5n-3	-0.1238	0.3567
22:5n-6	-0.0099	-0.0213
22:5n-3	-0.1435	0.2456
22:6n-3	-0.5234	0.5782

DHA (22:6n-3). The maximum loading on the PC2 was by DHA followed by EPA. DHA (22:6n-3) showed the maximum loading on PC2 followed by EPA (20:5n-3). The maximum negative loading on PC2 was by vaccenic acid or asclepic acid (18: ln-7), followed by palmitoleic acid (16: ln-7) (Table 3).

Many researchers have investigated that the composition of fatty acids in fish tissue is impacted by the constituent of the fatty acids in the diet. Viga and Grahl-Nielsen (1990) revealed that the richer the tissue in triacylglycerides, the closer is the resemblance of its fatty acid composition with that of the diet. The composition of fatty acids in phospholipids is generally expected to be more sensitive to the diet when compared with the composition of the fatty acids in the triacylglycerides (Joensen and Grahl-Nielsen, 2004). Joensen *et al*. (2000) studied stock variations in Faroe stocks of cod in which fatty acid composition in heart tissue, were made up of between 80% and 90% phospholipids. In terms of differences in fatty acid composition, the heart tissue was the most suitable for differentiation among fish species as tissue and oils were investigated (Joensen and Grahl-Nielsen, 2000, 2001). It was reported that the amounts of fatty acids in the populations of white sardine of four locations were different in most cases. The dominant saturated fatty acids that are found naturally in animal fats including fish lipids are 16:0 and 18:0. Although a range of chains from C12 to C24 can be found (Tocher, 2003). In the present study, the highest percentage showed in palmitic acid (16:0) in the samples of all the locations followed by stearic acid (18:0) ranged from 14.94% in Kolkata samples to 27.12% in Chennai samples. The most obvious trend in the results is the difference between the fatty acid composition of southern and northern populations along both coasts. The trend was more obvious in the case of SAFAs. All of the eight SAFAs showed a significant difference between any of the northern and southern populations, high content of SAFA was reported in the southern population. Meanwhile, the differences in PUFA were to a lesser extent with the northern population having a higher content of PUFA. This variation can be attributed to being the latitudinal effect on the saturated and unsaturated fatty acid content in fish tissues. Environmental temperature affects the fatty acid composition of the body of the poikilothermic fish, the degree of unsaturation increases by decreasing temperature (Morris and Culkin, 1989). There was a general tendency for retention of the n-3 FAs to be higher in the fish living at the lower temperature and it may be a reflection of a thermal acclimation response. (Bendiksen and Jobling, 2003 and Armstrong *et al*., 1994) studied the effects of season and location of catch on the fatty acid compositions of some Australian fish species. They observed that the highest n-3 PUFA content lipids were found in fish from colder waters. When fish are exposed to low temperatures a usual biochemical response is the increase in the unsaturation of the fatty acids incorporated into both the cell membrane lipids and the storage TAGs (Cossins and Lee, 1985; Hazel and Williams, 1990; Fodor *et al*., 1995; Logue *et al*., 2000; Jobling and Bendiksen, 2003; Hsieh *et al*., 2003, Sajina *et al.,* 2015).

CONCLUSION

The results of the present studies showed the northern and southern populations in *E. thoracata* along both coasts. It

revealed that there is the existence of different trends in populations of this species at the respective sampling locations. As Mumbai and Kolkata of northern populations are geographically distant, they cannot be considered a single stock, at the same time, the similarity between Cochin and Chennai samples indicated the occurrence of a single stock in the southern regions, the result of the present studies can be focused and extended further on delineating the populations from all the geographical locations along the Indian coast.

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