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Seasonal Variations of Fatty Acid Contents of *Saccostrea cucullata* at Intertidal Zone of Chabahar Bay

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Abstract: Seasonal variations of fatty acids were studied in the lipid fractions of the bivalve mollusk, *Saccostrea cucullata*, at the intertidal zone of Chabahar bay in the northern part of Oman Sea (Iran). Samples were collected in rocky shores between two stations. The analysis were carried by GC/MS chromatography. Thirteen fatty acids were identified, of which, the most important saturated fatty acids (SFA) were 14:0, 4, 8, 12 tri Me- 13:0, 16:0 and 18:0, the mono unsaturated fatty acids (MUFA) included 16:1n-9, 18:1n-9 and 20:1n-11, the polyunsaturated fatty acids (PUFA) were linoleic acid 9,12 18:2 , eicosapentaenoic acid EPA 20:5n-3 and arachidonic acid 20:4n-6.Variability of the fatty acid components were studied in four seasons. Maximum percentage level in *Saccostrea cucullata* for 14:0, 4, 8, 12 tri Me 13:0, 16:0 and 15:0 as saturated fatty acids was observed in summer, while for 18:1n-9, 20:1n-11 and 20:5n-3 (as unsaturated fatty acids) maximum concentration was observed in winter. The environmental factors were monitored monthly and their effects on seasonal variations of the fatty acids were studied by applying pearson coefficient correlation. The results showed the significant dependency of 20:1n-11 fatty acid concentration to ambient temperature and 9,12 18:2 fatty acid to silicate as environmental factors. Also, principal component analysis was done to establish the fatty acid groups. After Varimax rotation, three factors were extracted, of which first and second factors contributed to 86% of the data matrix. These were mainly dependent on the seasonal variations of the fatty acids.

Key words: Bivalve, lipid fractions, environmental factors, mollusk, gas chromatography

INTRODUCTION

Chabahar bay with northern 60E 37' 45" longitude and 27E 15' 45" latitude is located along the coast of Sistan Province in the South east of Iran, northern part of Oman Sea in Indian Ocean. This area posses one of the highest rates of primary productivity in the world due to seasonal upwelling (Passow *et al*., 1993; Barlow *et al*., 1999). This phenomenon provides a diverse and unique dietary selection for mollusks which in turn might result in different compositions of fatty acids. However, according to available data there is no earlier report about fatty acids in this region.

Investigations of fatty acids in marine mollusks have been carried out by Ackman *et al*. (1980), Ackman (2000), Joseph (1989), Dembitsky *et al*. (1993), Misra *et al*. (2002) and Abad *et al*. (1995) in many habitats. Among the mollusks, bivalves are more important because of their commercial value for use in human foodstuffs, the biological and pharmacological role of their polyunsaturated fatty acids and especially the fatty acid 20:5n3, which is useful in the treatment of some cardiovascular

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diseases (Joseph, 1982). Seasonal variations in lipid and fatty acid compositions of bivalves have been reported for several species including *Argopecten irradians*, *Tapes decusssatus*, *T. Philippinrum*, *Chlamys tehelcha*, *C. Opercularis*, *Venus pallina*, *Seapharca inequivalvis* and *Crassostrea virginica* (Barber and Blake, 1981; Beninger and Stephan, 1985; Kluytmans *et al*., 1985; Piretti *et al*., 1987, 1988; Chu *et al*., 1990). The composition of the lipid fraction of mollusks may be affected by external factors such as the changes of environmental conditions or internal factors such as sexual maturation (Piretti *et al*., 1988).

The purpose of this study was to provide information on fatty acids compositions of *Saccostrea cucullata*, class Bivalvia; order Pterioida; family Ostreidae and their seasonal variations with the aim of understanding their relationship with certain physico-chemical parameters of the environment.

MATERIALS AND METHODS

Oysters, with the same size (*S. cucullata*; 6-7 cm in length) to minimize compositional variations, were collected from Chabahar bay, in the rocky shores between two stations at a distance of about 7 km in four seasons (April, July, October 2007 and February 2008). The environmental conditions were monitored monthly from June 2007 until March 2008.

The whole body tissue of fifteen oysters were dissected from the shells in every sampling, frozen and stored in pre-weighted containers at -80°C for later analysis of the fatty acids. Wet fresh weight of the standard animal was 7 g. Five gram of each sample was taken, extracted using a homogenizer (Wagtech T1813) with a solvent mixture of chloroform/methanol 2:1 (v/v) and volume to weight ratio of 20:1. About 50 ppm of BHT (Butylated Hydroxy Toluene) was added as an antioxidant into the mixture (Jones *et al*., 1972). The total extract was filtered under vacuum using glass fiber filter (Whatman, S and S, GF6) and 0.5% NaCl (0.2 vol. of the extract) was added. The aqueous layer was re-extracted with chloroform. The combined organic layers were evaporated to about 3-5 cm^3 and then hydrolyzed with 5% aqueous KOH (20 cm^3) and methanol (100 cm^3) for 2-3 h at reflux temperature. After cooling, water (50 cm^3) was added and the basic solution was extracted twice with n-heptane/ diethyl ether 1:1 (v/v). The aqueous methanolic layer was acidified to pH = 2 and the fatty acids were extracted with n-heptane/diethyl ether 1:1(v/v, 3×100 cm^3). Then they were dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated to 2-3 cm^3 (Johns *et al*., 1980). The fatty acids were esterified with BF_3-methanol (Morrison and Smith, 1964) and heated in boiling water for 5 min. After cooling, 1 mL water and 2 mL pentane were added, vortexed (Stuart SA8) for 1 min and centrifuged (Heraeus Biofuge). Then the upper phase was collected. Pentane was evaporated and the residue dissolved immediately in 50-100 Fl of n-hexane for injection to the gas chromatograph. Each sample was treated and analyzed for three times.

The samples were injected into GC/Mass and the separation of the fatty acids methyl esters was performed using a Gas Chromatograph (Agilent Technologies, 6890) with a mass selective detector (6973N). GC/Mass analysis was done with an Electron Impact (EI) mode of 70 eV as ionization source and quadrupole mass filter with Chemstation data analysis system. The capillary column used was HP-5 (5% diphenyl 95% dimethyl siloxane copolymer) with 30 m length, 320 μm internal decimeter and 1 μm film thickness. The carrier gas was helium (purity , 99.999%). 0.5 μL of the extract containing the fatty acids methyl esters was injected into the injector using split mode with 50:1 split ratio. The injector temperature was 200°C, the detector temperature was 280°C and the oven temperature was programmed from 75°C min^{-1} to 270°C at 30°C min^{-1}, while the final temperature was held for 7 min (Casado *et al*., 1998).

To insure that all the components were detected, the final temperature was held for 20 min in the replicated test runs.

The fatty acids methyl esters were separated and identified in composition with the chromatograms of commercial fatty acids standards and in order to eliminate quantitative differences between the samples, the fatty acids composition was expressed in the relative terms, as a percentage of the total fatty acids. Peaks < 0.2% of the total area were not included in the profiles.

The pearson correlation coefficients and regression analysis were used in order to determine if there are relationships among the fatty acids variations and environmental parameters.

Principal Component Analysis (PCA) was performed to distinguish similarities between the fatty acids and categorizing them, on a personal computer using the statistical software package SPSS version 11.5. The data matrix for analysis consisted of the concentrations of identified fatty acids at four seasons. Factors were extracted by PCA. The factors which determined were extracted only in cases where the eigenvalues were over one. If the eigenvalue of the extracted factor was less than one, it was not considered in the data set. Then by rotation of axis (Varimax rotation), the factors treated mathematically for maximizing the load of factors and each factor was assumed to be independent.

RESULTS

The sea water conditions from June 2007 until March 2008 (the sampling period) are presented in Fig. 1. As shown in Fig. 1A with the advance of winter a decrease in water temperature was observed until reaching a minimum (21°C) in February and an increase of temperature during spring to summer until its maximum (33.07°C) in July. Chlorophyll-a showed relatively low values during the winter (Fig. 1B), with minimum concentrations (0.1 ppb) and increases in May, then shortly decrease and reach its maximum at fall (1.28 ppb). The trend of silicate and phosphate is slightly similar to each other, have their maximum levels in summer (Fig. 1C, E), as 0.6 and 0.504 ppm, respectively. Nitrate (Fig. 1D) has its minimum (0.050 ppm) in April and reach to its maximum (2.817 ppm) in May.

Increased differences in the salinity (Fig. 1F), were observed from May till September and in October has minimum of 36.43 PSU and then followed by a sharp increase in November, thereafter decreasing until February.

The analysis of fatty acid contents of *Saccostrea cucullata* during four seasons are reported in Table 1. Thirteen fatty acids were identified including seven saturated. The proportion of the saturated fatty acids varied from 64.135 to 86.211% of total lipids in *Saccostrea cucullata* (Table 2).

The major saturated fatty acids were 4,8,12-tri Me-13:0 , 16:0, 14:0 and 18:0. The monoenoics were 16:1n-9, 18:1n-9 and 20:1n-11. Linoleic acid 9,12- 18:2 was only identified in the dienoics group of the fatty acids. 20:4n-6 and 20:5n-3 were identified as tetra and penta enoic acids.

PCA, a multivariate technique with the aim of reduce the number of variables (measured fatty acids content in four seasons) to a smaller set of factors was applied to the fatty acids data set. The number of factors extracted from the variables was determined according to Kaiser's rule. This criterion retains only factors with eigenvalues that exceed one. The analysis of the fatty acid data established three factors. Varimax rotation for the correlations was applied. Concentrations of thirteen fatty acids as active variables in four seasons were selected. The cumulative variance among these three factors was 100%. The first factor was the most effective of the three factors and accounted for 60.134% of the variance (Table 3). Fatty acids with high factor loading values in the first factor were 14:0,4,8,12-tri Me-13:0, 15:0, 16:0, 17:0, 9,12- 18:2 , 18:1n-9, 18:0, 20:5n-3 and 20:1n-11. The second (PC2) and the third (PC3) explained 25.721 and 14.145% of the total variances, respectively. PC2 dominated by Me17:0 (0.929) and 20:4n-6 (0.95) and PC3 dominated by 16:1n-9 (0.945). Seasonal variations of fatty acids related to these three factors are represented in Fig. 2.

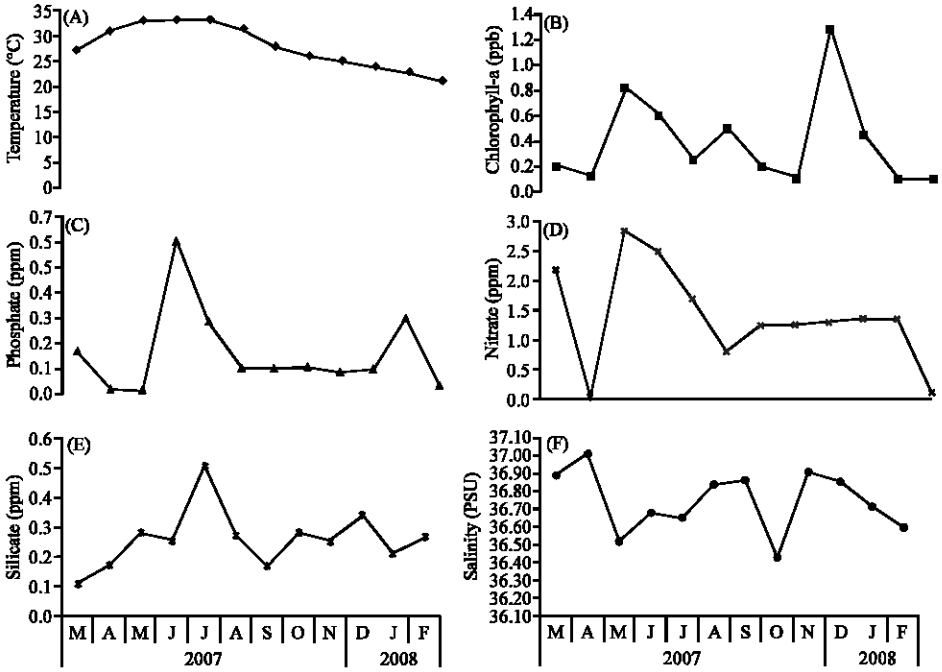

Fig. 1: Variations of environmental conditions in four seasons at Chabahar bay

Table 1: Fatty acid contents in *Saccostrea cucullata* during four seasons

Fatty acids	Spring (%)	Summer (%)	Fall (%)	Winter (%)
14:0	4.880	7.268	5.623	3.911
4,8,12-tri Me-13:0	1.098	2.099	1.006	0.468
15:0	1.613	3.868	2.288	0.904
16:1n-9	4.948	3.699	4.942	3.509
16:0	37.706	58.088	44.160	40.270
17:0	2.217	1.393	1.065	2.202
Me-17:0	4.910	3.336	6.622	5.227
9,12- 18:2	9.420	3.592	6.551	6.776
18:1n-9	7.863	2.394	4.053	8.412
18:0	11.711	10.159	10.999	12.180
20:4 n-6	1.977	0.863	3.519	2.324
20:5n-3	10.197	2.416	7.327	10.930
20:1n-11	1.460	0.825	1.846	2.887

Table 2: Total saturated fatty acid contents in *Saccostrea cucullata* during four seasons

Fatty acids	Spring (%)	Summer (%)	Fall (%)	Winter (%)
14:0	4.880	7.268	5.623	3.911
4,8,12-tri Me-13:0	1.098	2.099	1.006	0.468
15:0	1.613	3.868	2.288	0.904
16:0	37.706	58.088	44.160	40.270
17:0	2.217	1.393	1.065	2.202
Me-17:0	4.910	3.336	6.622	5.227
18:0	11.711	10.159	10.999	12.180
Total	64.135	86.211	71.763	65.162

The use of pearson correlation matrix among the environmental factors including salinity, temperature, chlorophyll-A, silicate, phosphate and nitrate (as nutrients), with fatty acids showed a strong negative correlation between the temperature and 20:1n-11 fatty acid (r = -0.97) and also for silicate and 9,12 18:2 fatty acid (r = -0.95).

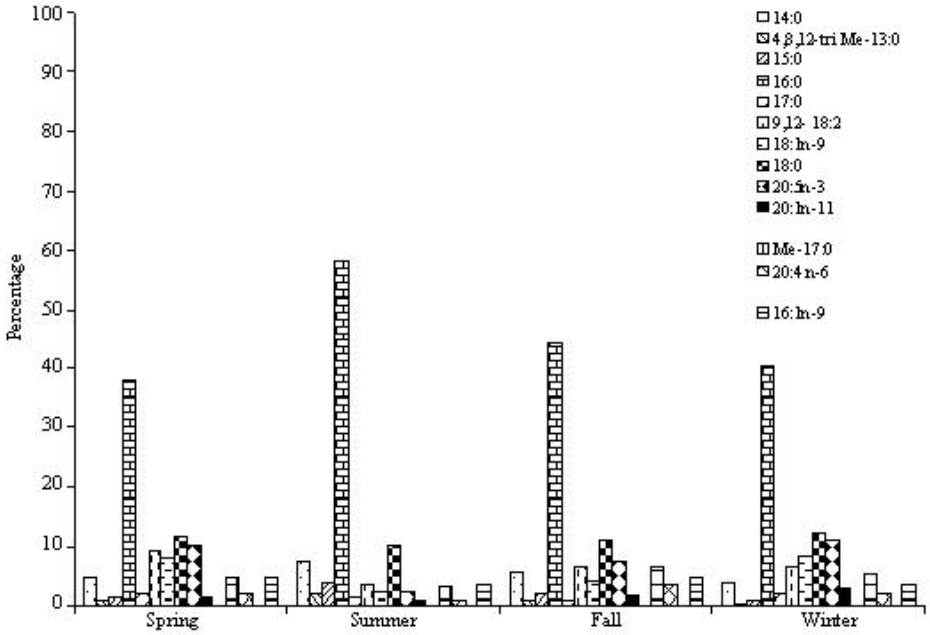

Fig. 2: Seasonal variations in the three groups of fatty acids of *Saccostrea cucullata*

Table 3: Factor analysis for three principal components (Varimax with Kaiser Normalization) for fatty acids in four seasons in Chabaharbay

Fatty acid	PC1	PC2	PC3
14:0	-0.930	-0.366	0.028
4,8,12-tri Me-13:0	-0.795	-0.605	0.057
15:0	-0.924	-0.381	-0.032
16:1n-9	-0.054	0.322	0.945
16:0	-0.843	-0.369	-0.391
17:0	0.913	-0.407	-0.030
Me-17:0	0.213	0.929	0.303
9,12-18:2	0.744	0.143	0.653
18:1n-9	0.970	0.044	0.071
18:0	0.967	0.254	-0.001
20:4 n-6	0.159	0.950	0.268
20:5n-3	0.928	0.329	0.176
20:1n-11	0.712	0.577	-0.402
Eigenvalue	9.128	2.487	1.385
Variance of (%)	60.134	25.721	14.145
Cumulative (%)	60.134	85.855	100.000

According to the results of pearson correlation, regression method applied for temperature and 20:1n-11 fatty acid; silicate and 9,12 18:2 fatty acid, which resulted $r^2 = 0.96$ and 0.91, respectively and indicate the important role of the above ecological parameters in the mentioned fatty acids variations.

DISCUSSION

From the above context, total saturated fatty acids varied between 64.135 and 86.211% in *Saccostrea cucullata*. Thirteen fatty acids including seven saturated, three monoenoics, one dienoic, one tetraenoic and one pentaenoic fatty acids were determined.

By applying PCA method fatty acids categorised in three groups, due to their similar trends. Based on PCA results, PC1 consist of the saturated fatty acids (14:0, tri Me-13:0, 15:0 and 16:0) which had their maximum level in summer and the unsaturated fatty acids (20:5n-3, 18:1n-9 and 20:1n-11) which had their maximum level in winter. This result confirm the earlier reports which have shown an inverse relationship between temperature and the amount of polyunsaturated fatty acids in tissue lipids of invertebrates and diminution of the saturated fatty acids during winter (Chu and Greaves, 1991).

Accumulation of polyunsaturated fatty acids (especially 20:5n-3) and minimum levels of saturated fatty acids during winter and reverse change in summer may be due to the adaptive regulation of melting point of cellular lipids (Pazos *et al*., 1996). Also, tri Me 13:0 (4,8,12 tri Methyl Thridecanoic acid) could be originated from the bivalves diet (Wood, 1974; Johns *et al*., 1979) and related to composition of the food sources which is abundant in summer in the sampling area. Considering the fact that 20:5n-3 could not be synthesized de novo (De Moreno *et al*., 1980) and is also related to bivalves diet, maximum percentage level of tri Me 13:0 in summer and 20:5n-3 fatty acids in winter could be originated from different diet sources.

Also 18:0 fatty acid in PC1 has a slightly constant trend throughout the year, which is in line with other similar researches regarding bivalves (De Moreno *et al*., 1980). In addition to other parameters in PC1, 9,12 18:2 fatty acid reached to its maximum level in spring which in this time the available food source from phytoplankton origin gives an increase in polyunsaturated fatty acids (Langdon and Waldock, 1981).

PC2 consists of Me17:0 and 20:4n-6 fatty acids which have their maximum in fall. These levels probably arise from selective retention of the 20:4n-6 fatty acid for use in reproductive processes and it has been shown that the 20:4n-6 fatty acid in the oyster can be implicated in the synthesis of various neurotransmitters related to the reproductive processes, such as the prostaglandins 2 (Freites *et al*., 2002). Besides 20:4n-6 might be used as an energy reserve during periods of food scarcity (Gardner and Riley, 1972). 16:1n-9 fatty acid had been categorized in an individual group (PC3) which reached its maximum level in spring, decreased toward summer; thereafter increased in fall slightly similar to spring and decreased in winter again similar to summer. Therefore, it had a unique trend among the others which categorised in a separate group. Also its maximum in spring confirms again the research of Langdon and Waldock (1981), about increasing such polyunsaturated fatty acids in this time in relation to available food source from phytoplankton origin.

According to pearson correlation, temperature among ecological factors showed a significant correlation with the 20:1n-11 fatty acid, i.e., with the increasing of temperature, the amount of fatty acid will decrease with a strong correlation and a good regression which is mentioned before that unsaturated fatty acids must be increased in low temperatures because of their higher melting point compared to the saturated fatty acids and reflect that these fatty acids could be used in metabolism of cells.

A significant negative correlation was observed between silicate and 9,12 18:2 fatty acid. This can be explained by the fact that in high temperatures a decrease occurs in silicate availability and highly saturated fatty acids, which will increase unsaturated ones (Mortensen *et al*., 1988). Similar results had been observed that within a silicate deprivation diet, 18:2n-6 fatty acid increased in fatty acid composition of sea Scallop *Placopecten magellanicus* larvae (Pernet and Tremblay *et al*., 2004).

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