

Temperature dependence of heptadecanoic and methyl-heptadecanoic fatty acids of *Chiton lamyi* in Chabahar Bay

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Abstract

Chiton lamyi species collected from the intertidal zone of Chabahar bay analyzed seasonally for its fatty acid compositions in foot. Thirteen fatty acids identified in both sections by gas chromatography/mass spectroscopy. Major unsaturated fatty acids were palmitoleic acid, oleic acid and 11-eicosenoic acid and PUFAs included linoleic acid, eicosapentaenoic acid and arachidonic acid. Effects of monthly temperature and nutrients (silicate, phosphate and nitrate) investigated to detect seasonal variations of fatty acids. Pearson analysis results showed correlation among palmitic and oleic acids with silicate and phosphate; linoleic and arachidonic acids with nitrate in *Chiton lamyi* internal tissues, but no correlation observed in foot. Heptadecanoic and methyl- heptadecanoic acids contents and temperature correlated strongly in *Chiton lamyi* foot.

Keywords: *Fatty acids, Chiton lamyi, GC/MS, Environmental parameters*

1. Introduction

Fatty acids in marine invertebrates have been studied in many habitats because of their importance in human life (Ackman, 2000). (Ackman et al., 1980, Joseph, 1989) reported on studies regarding many mollusks. Species of the phylum mollusca contain important fatty acids and are of interest because of their variability in different areas (Kostetsky, 1985, Naumenko, 1987, Dembitsky, 1994).

Polyplacophora, one of the important classes of molusca has been studied for its feeding ecology and gut contents (Latyshev, 2004) and fatty acid in

Ponerplax costata (Johns et al., 1980).

Effects of environmental factors on fatty acids have not been studied extensively. Sanina (2002) studied thermotropic behavior of major phospholipids in marine invertebrates, and seasonal variations of fatty acids was carried out in flat oysters by Abad. et al., (1995) Seasonal changes in fatty acid compositions in a number of gastropods also has been studied by Calzolari et al., (1971). Chabahar bay is located along the coast of Sistan province, southeast of Iran and northern part of the Oman Sea in the Indian Ocean. Seasonal upwellings due to monsoonal currents in this area generate one of the highest rates of primary productivity in the world (Passow et al., 1993, Barlow et al., 1999). This phenomenon provides a diverse and

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unique dietary selection for mollusks, which in turn might result in different compositions of fatty acids. There are no report regarding compositions of fatty acids in foots of mollusks in this region. In this paper, compositions of fatty acids and their seasonal variations in foot of polyplacophora *Chiton lamyi* are determined quantitatively. This study was the first investigation undertaken in foot of this species in the Chabahar Bay, a region amongst the highest known primary productivity ecosystems of the world. Correlations of seasonal fatty acid components with temperature and nutrients (phosphate, nitrate and silicate) were also examined.

2. Materials and Methods

Thirty equall size adult *Chiton lamyi* individuals were collected from rocky shores in every sampling at Chabahar bay in April, July, October 2007 and February 2008. Sampling location coordinates were 60°37'45" longitude and 27°15'45" latitude, distance between two sampling stations was about 7km and water was 3-3.5m deep. Temperature and nutrients were measured monthly in sampling sites concurrently. Samples were transferred to the lab immediately. Foot dissected from individual samples, weighed and was frozen to -18°C for further experiments. Extraction of samples carried out using a homogenizer (Wagtech T1813) for containing a solvent mixture of chloroform/ methanol 2:1 (v/v) and volume to weight ratio 20:1(v/w). Fifty ppm of BHT (Butylated Hydroxy Toluene) were added as an antioxidant to prevent oxidation of unsaturated fatty acids (Jones et al., 1972). The total extract filtered under vacuum using glass fiber filter (Whatman, S&S, GF6) and then 0.25g NaCl was added for more efficiency of extraction. The aqueous layer was re-extracted with chloroform. The volume of combined organic layer was reduced by rotary evaporator to about 3-5 ml. Hydrolysis of fatty acids was carried out with 5% aqueous KOH (20ml) and methanol (100ml) for 2-3 hours at reflux temperature. After cooling,

distilled water (50ml) 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 fatty acids was extracted three times with 50ml of n-heptane / diethyl ether 1:1(v/v) and was dried out with anhydrous MgSO₄ then, extract was filtered and the filtrate was reduced to 2-3ml (Johns et al., 1980). Fatty acids esterified with solution of 14% BF₃-methanol (Morrison et al., 1964) and heated in boiling water for 5 minutes. After cooling, 1 ml water and 2 ml pentane added to the sample, vortexed (Stuart SA8) for 1 minute, centrifuged (Heraeus Biofuge) and the upper phase was collected. Pentane was evaporated and the residue was dissolved immediately in 50-100 µl n-hexane. This solution was used to inject to gas chromatograph. Each sample was treated and analyzed in triplicates.

Separation of FAMES was performed by a GC/Mass apparatus (Agilent Technologies, 6890) equipped with a mass selective detector (6973N).

GC/Mass analysis was performed with electron impact (EI) mode of 70eV as ionization source and quadrupole mass filter with Chemstation data analysis system. The capillary consisted of a 30m long, 320 µm internal decimeter and 1 µm film thickness HP-5 (5% Diphenyl 95% Dimethyl siloxane copolymer) column . Carrier gas was helium (purity 99.999%).

Extract (0.5 µL)containing fatty acids methyl esters was injected in the injector using split mode with 50:1 split ratio. Injector temperature was 200°C, detector temperature was 280°C, oven temperature was programmed from 75°C/min to 270°C at 30 °C/min and the final temperature was held for 20 minutes in replicated test runs to insure detecting all components (Casado et al., 1998).

Fatty acid methyl esters identified comparing the obtained peaks with chromatograms of commercial fatty acid standards. The Pearson correlation coefficients were applied to study the relationships among fatty acids variations and environmental parameters. In addition, regression analysis was used

for the components, showing strong correlations based on Pearson analysis, in order to predict the effects of each environmental factor as independent variable, on fatty acids as dependent.

Pearson analysis applied in order to predict the effect of environmental factors as independent variable, on fatty acids composition. In addition, regression analysis used for the components, which resulted strong correlations in.

3. Results

Seasonal variations of temperature and nutrients from June 2007 until March 2008 presented in Fig 1. Water temperature varied from a minimum of 21°C in February to a maximum of 33°C in July (Fig 1a). Silicate and phosphate followed almost similar trends rising to their maximum levels in summer as 0.6 and 0.50 mg/l, respectively (Figs 1b & c). Nitrate displayed a minimum of 0.05 mg/l in April and a maximum of 2.81 mg/l in May (Fig 1d).

Fatty acid components found in *Chiton lamyi* foot were the same as those found in its internal tissues (Table 1). As such, palmitic acid was the major

saturated fatty acid (varying in concentration from 27.44% in winter to 38.52% in fall) and oleic acid was the major unsaturated one (varying from 12.80% in fall to 25.76% in winter). Saturated and unsaturated fatty acids concentrations varied from 44.59% to 59.64%) and 40.35% to 55.40%, respectively. The highest quantities of saturated and unsaturated fatty acids were observed in fall and in winter, respectively. Consequently, no similarity was observed in proportional quantities of total saturated and unsaturated fatty acids in internal tissue and foot of *Chiton lamyi* during the experiment.

Figure. 2 represents seasonal variations in unsaturated fatty acids contents in foot of *Chiton lamyi*. Linoleic and palmitoleic acids showed similar variation trends throughout the year in *Chiton lamyi* foot, with minimum and maximum quantities in winter and summer, respectively (Fig 2). Oleic and eicosapentaenoic acids showed similar trends in *Chiton lamyi* foot (Fig. 2) but the minimum and maximum quantities occurring in winter and summer as well. This similarity in trends for the two fatty acids could be a common criteria for monitoring seasonal variations of fatty acids in internal tissue and foot of *Chiton lamyi*.

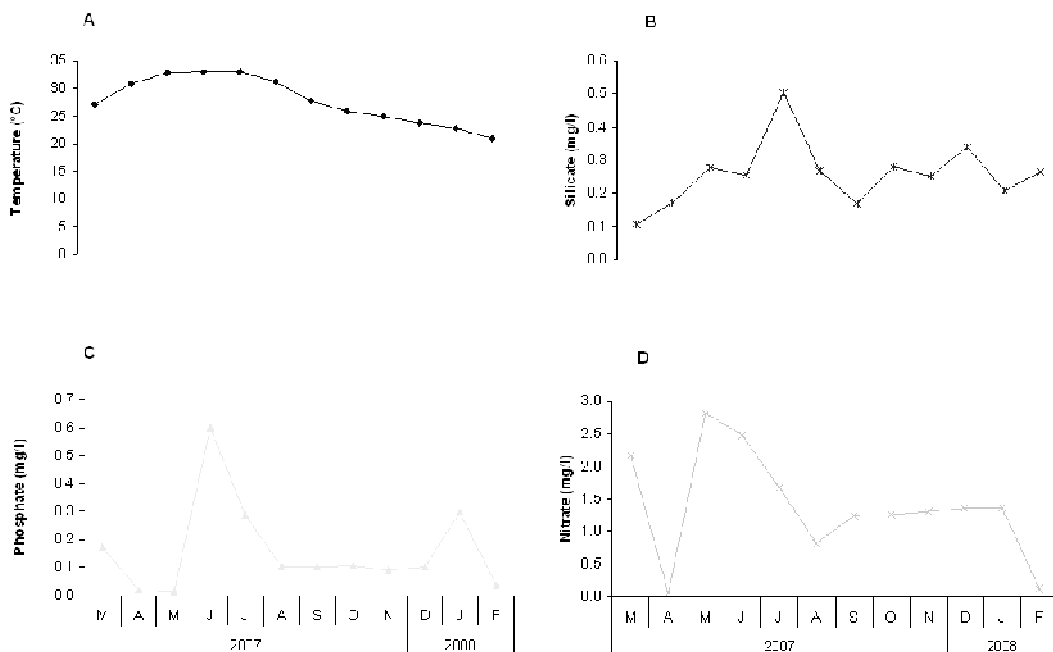


Fig 1. Variations of environmental parameters in four seasons at Chabahar bay

Table 1- Total unsaturated fatty acid contents in *Chiton lamyi* foot

Fatty Acids	Spring	Summer	Fall	Winter
4,8,12-tri Me-13:0	0.794%	1.156%	1.101%	1.189%
16:1n-9	9.573%	10.874%	9.723%	4.103%
Me-17:0	1.378%	1.260%	2.347%	3.483%
9,12- 18:2	12.662%	14.434%	14.211%	10.363%
18:1n-9	19.119%	16.116%	12.808%	25.760%
20:4 n-6	0.958%	1.636%	0.700%	5.114%
20:5n-3	1.811%	1.539%	0.538%	3.280%
20:1n-11	2.196%	1.733%	2.372%	6.783%
%Total Unsaturated	46.319%	46.332%	40.352%	55.403%

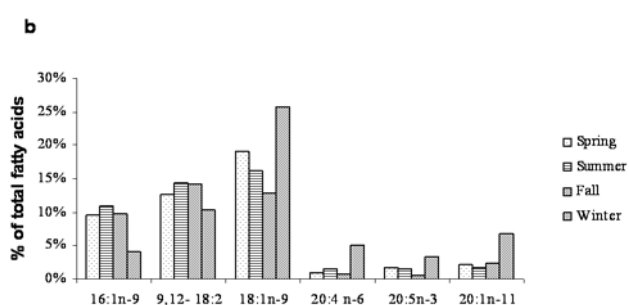


Fig 2. Seasonal variations of unsaturated fatty acids of *Chiton lamyi* foot

In order to study the correlation between seasonal variations of fatty acids and environmental parameters (temperature and nutrients such as silicate, phosphate and nitrate), Pearson correlation statistics applied for both foot of *Chiton lamyi*. Correlation coefficient matrix for temperature and 13 fatty acids of *Chiton lamyi* foot, showed strong negative correlation between 17:0 and Me17:0 fatty acids and temperature ($r=-0.99$ and -0.98) (Table 2).

Table 2- Regression analysis for temperature and Me 17:0 Fatty acid

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error			
1 (Constant)	.092	.011		8.691	.013
Temp	-.003	.000	-.979	-6.739	.021

Dependent Variable: Me 17:0 Fatty acid
 $r^2 = 0.95$

This means that any increase in temperature would decrease the amount of fatty acids Pearson analysis did not show significant correlations between other fatty acids of *Chiton lamyi* foot with temperature.

Because of strong correlation between 17:0 and Me17:0 fatty acids with temperature, univariate regression analysis was applied by considering temperature as independent variable and each fatty acid as dependent. Results of this study showed r^2 values of 0.98 and 0.95, implying strong effect of temperature on amounts of mentioned fatty acids (Tables 3). Using Tables 3, future amounts of fatty acids could be predicted applying following regression equation:

$$Y = a + b_1x_1 + b_2x_2 + b_3x_3 + (Eq. 1)$$

Where **Y** represented unstandardized coefficient (amount of fatty acid); parameter **a**, a constant derived from the table; parameter **b**, efficiency coefficient also calculated in the table and parameter **x**, temperature.

Table 3- Regression analysis for temperature and 17:0 Fatty acid

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error			
1 (Constant)	.043	.002		17.377	.003
Temp	-.001	.000	-.994	-12.727	.006

Dependent Variable: 17:0 Fatty acid
 $r^2 = 0.98$

Pearson analysis was also performed on the nutrients and the fatty acids of foot of *Chiton lamyi*. No significant correlation was found from primary results between seasonal variations of fatty acids and nutrients for the foot samples. Secondary results were derived from analyzing seasonal fluctuations in amounts of each nutrient with percentages of seasonal changes in fatty acids for internal body tissue of *Chiton lamyi*. Resulting matrices showed significant correlations for nitrate with linoleic ($r = -0.980$) and arachidonic ($r = -0.96$) acids, silicate with palmitic ($r=0.95$) and oleic ($r=-.96$) acids and phosphate with oleic acid($r=-0.96$). Considering the obtained r-values, univariate regression analysis was applied. From r^2 values for linoleic (0.96) and arachidonic (0.93) acids as dependent variables, and related regression tables prediction, the effectiveness

of nitrate as independent variable could be possible as mentioned above (equation 1). Similarly, r^2 for palmitic (0.90) and oleic (0.98) was derived in relation to silicate. R^2 value for phosphate and oleic acid was 0.93. Considering oleic acid the common component for phosphate and silicate in Pearson analysis, bivariate regression analysis was applied for oleic acid as dependent in relation to phosphate and silicate as independent variables. This analysis did not show a significant value. It concluded that each environmental parameter could be effective on fatty acids contents in unity but ineffective in conjunction with other parameters.

4. Discussion

Fatty acid components of internal body tissues and foot of *Chiton lamyi* were similar. but differed in proportion. Misra, (2002) studied variation in amounts of fatty acids in different organs of other mollusks. In the case of *Chiton lamyi*, although, there were some dissimilarity in amounts of fatty acids in internal body tissues and the foot. This difference might be because of different physiological adapting ability of organs exposed to ecological circumstances and thus producing different amounts and different kinds of fatty acids. 4, 8, 12-tri methyl-13:0 fatty acid frequented more abundantly in internal body tissue of *Chiton lamyi*, with a maximum level in spring. 4, 8, 12 trimethyl tridecanoic acid (4, 8, 12 TMTD) originated phytol and chloroplast membrane lipids and was dependent on chiton's diet (Wood, 1974; Johns et al., 1979). The highest level of unsaturated fatty acids in *Chiton lamyi* foot was observed in winter, while in the internal body tissues it was achieved in fall. Other researchers had reported an inverse relationship between temperature and the amount of polyunsaturated fatty acids in tissue lipids of invertebrates (Chu et al., 1991, Pazos et al., 1996), because of the adaptive regulation of melting point of cellular lipids. Results of this study showed,

however, that the difference in the case of internal tissues and foot of *Chiton lamyi* is probably due to the adapting capability of different organs to climatic conditions, such as temperature changes .

Seasonal variations of fatty acids such as 17:0, Me17:0 and 18:0, had similar trends throughout the research period in internal tissue of *Chiton lamyi*. This result was observed in *Chiton lamyi* foot as well. Unsaturated oleic and eicosapentaenoic fatty acids had similar seasonal variation trends in foot and internal tissues. Therefore, it was clear that all fatty acid components did not have similar patterns of variation throughout the year, neither in an individual tissue nor between different organs.

Two important environmental factors of temperature and nutrients (food availability) are reported to alter tissue lipid levels of invertebrates. This, together, might be contributed to seasonal variations in fatty acid compositions of *Chiton lamyi*; To test this, Pearson analysis was done for fatty acids in relation to temperature and nutrients (silicate, phosphate and nitrate) as environmental factors. Components which showed correlation with temperature were 17:0 and Me17:0 fatty acids, only in foot. Therefore, temperature could have further effects in fatty acids of the organs more exposed to external environmental conditions depending on the structure and physiology of those organs. In the case of nutrients, they had correlations with a few fatty acids of internal tissues and for the *Chiton lamyi* foot, there was no correlation. This might be related to the role of these nutrients in metabolism of internal organs more than that in foot. Accumulation of total lipids in phytoplankton cells under nutrient (silicon, phosphorus and nitrogen) depletion was investigated by (Lombardi & Wangersky, 1991, Parrish et al., 1990). Also according to other researches (Sterner, 1994), mineral limitation of aquatic herbivores, appear frequently to be limited by the quantity of mineral elements in their food. Therefore, it is possible that nutrients in the feeding regime affect fatty acid contents of mollusks directly.

Since little is reported on lipids of chitons, especially for the species of Chabahar bay area, this research provided useful information for other researchers interested in this field.

It is emphasized that fatty acid contents of foot of *Chiton lamyi* were similar, but there was difference between their fatty acid fluctuation trends in different seasons. Therefore, it was concluded that fatty acid variations were not the same at different organs of a mollusk. Among ecological factors, it was noticed that temperature and nutrients produced results countering foot of *Chiton lamyi*. Some fatty acids in foot were affected strongly by temperature.

In general, it was concluded that was a relative relationship between temperature and nutrients and fatty acids variations in this species. This might be because other factors such as chlorophyll-a, content of food supply or internal factors such as reproduction cycle could also affect fatty acids contents and composition. In addition, the significant correlation between temperature and Me 17:0 and 17:0 Fatty acids shows the increase of these fatty acids is a function of temperature raise. This is believed to be due to the metabolic rate increase in warmer seasons and the better yield of energy for saturated fatty acids that is a usual phenomenon in other animals. Therefore nutritional use of the foot tissues is recommended to be performed at colder seasons.

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