شناسایی ترکیبات اسید چرب و مطالعه اثرات عوامل محیطی بر تغییرات فصلی این ترکیبات در دوگونه از شکم پایان غالب در خلیج چابهار

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تاریخ دریافت: ۱۳۹۰/۰۵/۱۳ تاریخ پذیرش: ۱۳۹۰/۰۸/۳۰

چکیده

ترکیبات اسید چرب و تغییرات فصلی این ترکیبات در دو گونه از شکم پایان غالب منطقه خلیج چابهار واقع در جنوب شرقی ایران و شمال دریای عمان ، شامل نریتا تکستیلیس و توربو کوروناتوس شناسایی و بررسی شد. سپس عوامل محیطی شامل دما ، کلروفیل a و شوری به طور ماهانه اندازه گیری و اثرات آن ها در تغییرات ترکیبات اسید چرب توسط روش های آنالیز آماری بررسی شد. توسط روش کروماتوگرافی گازی ۱۲ اسید چرب در نریتا تکستیلیس و ۱۵ اسید چرب در توربو کوروناتوس شناسایی شد. بر طبق نتایج کروماتوگرافی گازی در نریتا تکستیلیس اسیدهای چرب غیر اشباع غالب بر اسیدهای چرب اشباع شده بودند و اسید اولییک اسید چرب غالب در این جاندار بود در حالیکه در توربو کوروناتوس اسیدهای چرب اشباع شده غالب بوده و اسیدچرب اصلی اسید پالمیتیک بود. نتایج آنالیز آماری ارتباط معنی داری بین اسید اولییک و دما ، اسید گادولییک و کلروفیلa و اسید مارگاریک با شوری را در نریتا تکستیلیس نشان داده و در توربو کوروناتوس شوری تنها فاکتور محیطی بوده است که ارتباط معنی دار با اسید لیگنوسریک نتیجه داده است. در نتیجه گیری کلی می توان گفت که محتویات اسید چرب در جانداران متفاوت بوده و همچنین فاکتورهای محیطی اثرات متقاوتی بر تغییرات جانداران در دوگونه از یک رده و حتی در یک زیستگاه مشترک نشان می دهد.

واژگان کلیدی: اسیدهای چرب، کروماتوگرافی گازی، شکم پایان، فاکتورهای محیطی، تغییرات فصل

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Identification of fatty acid contents and study the effects of environmental factors on their seasonal variations in two dominant gastropods of Chabahar bay

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Abstract

Fatty acid contents and their seasonal variations studied in *Nerita textilis* and *Turbo coronatus*, two dominant gastropods of Chabahar bay, in the South east of Iran and northern part of Oman Sea in Indian Ocean. This area provides high rates of primary productivity and so a diverse food source for mollusks. Environmental factors, including temperature, chlorophyll a and salinity measured monthly and their effects on fatty acids variations considered by statistical analysis. By Gas Chromatography twelve and fifteen fatty acids identified in *Nerita textilis* and *Turbo coronatus*, respectively. Unsaturated fatty acids dominated over saturated ones in *Nerita textilis* and oleic acid was the major fatty acid, while saturated fatty acids were dominants in *Turbo coronatus* and the major fatty acid was palmitic acid. Statistical analysis showed correlation of oleic acid with temperature, gadoleic acid with chlorophyll a and margaric acid with salinity in *Nerita textilis* , while salinity was the only environmental factor which showed correlation with lignoceric acid in *Turbo coronatus* . In conclusion it could be finding that the fatty acid contents might be different and also, there could be different effects of environmental factors on fatty acid variations in two species of a class in a common habitat.

Keywords: fatty acids, gas chromatography, gastropods, environmental factors, seasonal changes

Introduction

Chabahar bay, in the northern part of Oman Sea in Indian Ocean and south east of Iran, provides high rates of primary productivity and so a diverse food source for mollusks in this area (Barlow *et al*., 1999).

Fatty acid contents in mollusks are studied in many habitats, because of their importance in human's life (Akhman *et al* ., 2000 ; Joseph, 1989 ; Dembitsky *et al* . , 1993 ; Misra *et al* ., 2002; Abad *et al* . ,1995).

The effects of environmental factors on the fatty acids is insufficient, however thermo tropic behavior of major phospholipids in marine invertebrates (Sanina & kostetsky, 2002) and seasonal variations of fatty acids in flat oysters were studied (Abad *et al*., 1995).

There is no previous report about fatty acid components of mollusks in Chabahar bay. In this research fatty acid compositions and their seasonal variations of two dominant gostropods, *Nerita textilis* and *Turbo coronatus*, studied for the first time in this area. Also, the effects of environmental factors on the fatty acids variations followed by Pearson statistical analysis and evaluated the intensity and direction of the effects of those factors on fatty acids.

Materials and Methods

About 100 *Nerita textilis* and 80 *Turbo coronatus* were collected in every sampling at intertidal zone of Chabahar bay , with Northern 60°37′45″ longitude and 27°15′45″ latitude between two stations at the distance of about 7 km , where the water depth was 3 – 3.5m . Samples collected with the same size in four seasons at April, July, October 2007 and February 2008. Temperature, Chlorophyll a and Salinity measured monthly in the collecting site at the same time.

After sampling the species transferred to the lab immediately. Whole body tissue dissected from the shells, weighed and froze to -18°C for further experiments. Fatty acids extracted from gastropods tissues by (Johns *et al.,* 1980) method. The fatty acids esterified (Morrison & Smith, 1964) and the solution made ready for injection to the gas chromatograph. Each sample was treated and analyzed for triplicates.

Separation of fatty acid methyl esters was performed by GC apparatus made up by Agilent Technologies (6890) with a FID detector. The column used was BP-70 with 120m length, 250mm internal decimeter and helium gas and 0.5 ml of extract was injected. Injector temperature was **250°C,** detector temperature was **250°C** and oven temperature programmed from 198°C at 7/46 minutes and raised 5°C min^{-1} to 220°C and held in this final temperature for 70 minutes.

Fatty acid methyl esters identified by comparing the obtained peaks with the chromatograms of commercial fatty acid standards. The Pearson correlation coefficients were applied for study the relationships among the fatty acids variations and environmental parameters. In addition, regression analysis applied for the components which resulted strong correlations of Pearson analysis, in order to predict effectiveness of each environmental factor as independent variable, on fatty acids as dependent.

Results

The seasonal variations (from June 2007 until March 2008) of temperature, chlorophyll a and salinity are shown in Fig.1. As shown in Fig.la, water temperature was in minimum of 21°**C** in February and reached to its maximum of 33°**C** in July. Chlorophyll a (Fig.lb) had its maximum (0.53ppm) in fall and its minimum in winter (0.28ppm) which could be related to nutrients and phytoplankton loads of the area. Salinity (Fig.lc) had a slightly constant trend with the lower amount of 35.55 PSU in spring.

Table 1 shows twelve fatty acids identified in whole body tissue of *Nerita textilis* , which the major saturated ones were myristic , palmitic , stearic and behenic acids . The monoenoics were palmitoleic , oleic , vaccenic and gadoleic acids and the other poly unsaturated ones were linoleic , alpha linolenic and eicosapentaenoic(EPA) acids.

Table 1 Fatty acid compositions(%) of *Nerita textilis*

Fatty Acids	Spring	SD	Summer	SD	Fall	SD	Winter	SD
C14:0	3.61	0.76	5.89	0.09	3.48	0.66	2.25	0.09
C16:0	20.78	0.99	26.10	0.87	17.83	1.33	18.62	0.88
C16:1	3.59	0.12	3.60	0.89	2.28	1.55	2.85	0.09
C17:0	1.28	0.32	1.19	1.54	1.21	0.09	1.21	0.05
C18:0	7.42	0.98	9.13	1.44	7.35	0.78	8.49	0.55
C18:1t	4.50	1.23	3.40	0.07	2.9	0.67	2.60	0.77
C18:1c	24.90	1.87	20.03	0.65	30.33	0.55	33.06	0.05
C18:2c	10.16	0.98	7.55	1.53	12.34	1.44	12.57	0.44
C18:3 Alpha	4.96	0.03	1.56	1.66	4.16	1.34	3.16	0.54
C20:1	1.42	0.54	1.76	0.96	2.46	0.77	1.04	1.12
C22:0	2.78	0.09	2.24	0.55	3.67	0.01	2.92	0.88
C20:5	2.62	0.04	3.75	1.22	3.02	0.02	2.89	0.05

The major fatty acid was oleic acid and the proportion of unsaturated fatty acids varied from 55.45% in summer to 66.51% in winter which dominated over saturated fatty acids.

The maximum percentage level of saturated fatty acids was in summer (44.55%) and the minimum was in winter (33.49%).

Figure 2 shows the seasonal variations of fatty acids during four seasons in *Nerita textilis* .

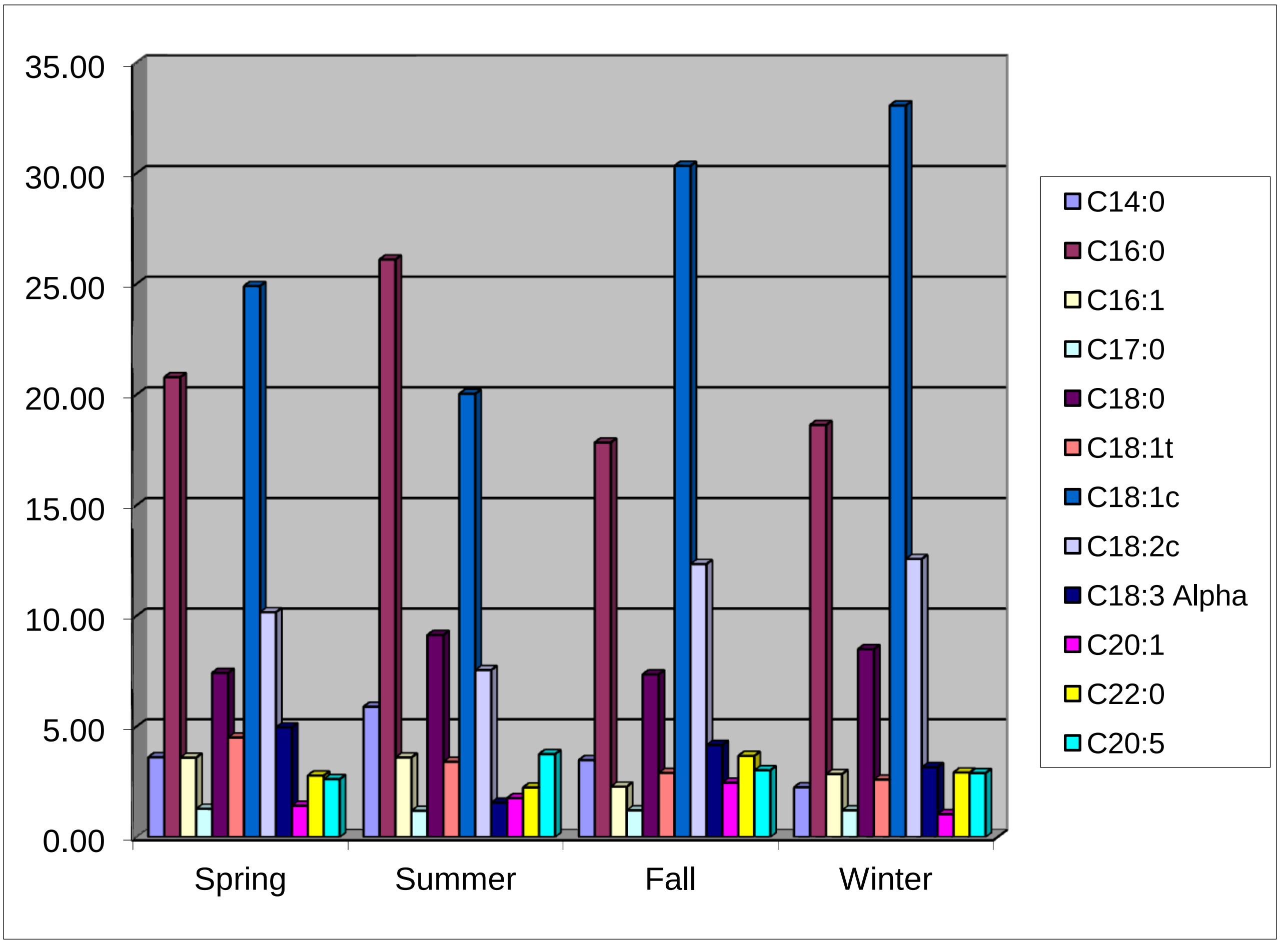

Figure 1- Variations of environmental parameters in seasons in Chabahar Bay.

According to Fig.2, variation trends of palmitic, stearic and eicosapentaenoic acids are similar and have their maximum in summer while linoleic and alphalinolenic acid have their maximum in winter.

Fatty acid compositions of *Turbo coronatus* are shown in Table 2. There are fifteen fatty acids identified, including seven saturated and eight unsaturated fatty acids, which the major saturateds were myristic, palmitic, stearic, behenic, tricosanoic and lignoceric acids.

Table 2 - Fatty acid compositions(%) of *Turbo coronatus*

Fatty Acids	Spring	SD	Summer	SD	Fall	SD	Winter	SD
14:0	5.01%	1.11	1.91%	0.99	2.01%	0.88	2.98%	0.05
16:0	24.61%	0.09	17.97%	1.55	24.88%	1.74	18.85%	0.68
16:1n-9	5.64%	1.23	2.66%	0.09	1.68%	0.09	3.96%	0.18
17:0	2.46%	1.33	1.42%	1.77	1.02%	0.02	1.48%	1.19
18:0	5.02%	0.08	2.02%	0.08	8.04%	0.73	6.79%	0.28
18:1t	0.43%	0.78	0.78%	0.55	11.51%	0.55	1.93%	0.05
18:1c	15.48%	0.88	18.39%	0.75	30.26%	0.33	27.29%	0.93
18:2c	6.02%	0.03	7.76%	1.12	9.93%	0.86	3.12%	1.68
18:3 alpha	2.57%	0.44	1.18%	0.09	1.22%	0.64	2.49%	0.17
20:1	2.49%	0.75	0.42%	0.04	2.02%	0.19	4.16%	0.59
22:0	10.12%	0.66	1.16%	1.45	2.37%	1.16	4.82%	0.44
20:5	6.64%	1.78	1.25%	1.33	2.88%	0.95	7.12%	0.14
23:0	4.71%	0.72	0.41%	0.03	0.37%	0.07	2.16%	1.21
24:0	2.37%	1.84	0.35%	0.54	0.52%	0.38	0.95%	0.46
22:5	3.51%	0.77	0.20%	1.31	0.34%	0.77	1.88%	0.03

The major unsaturated fatty acids were palmitoleic, vaccenic, linoleic, alphalinolenic, gadoleic, EPA and docosopentaenoic acids. Palmitic acid was the major fatty acid and saturated fatty acids were dominated over unsaturated ones with the maximum percentage level of 99.05% in fall and minimum of 57.88% in summer.

The maximum percentage level of unsaturated fatty acids was in summer (42.12%) and the minimum was in fall (0.95%).

Seasonal variations of fatty acid compositions of *Turbo coronatus* are shown in Figure 2.

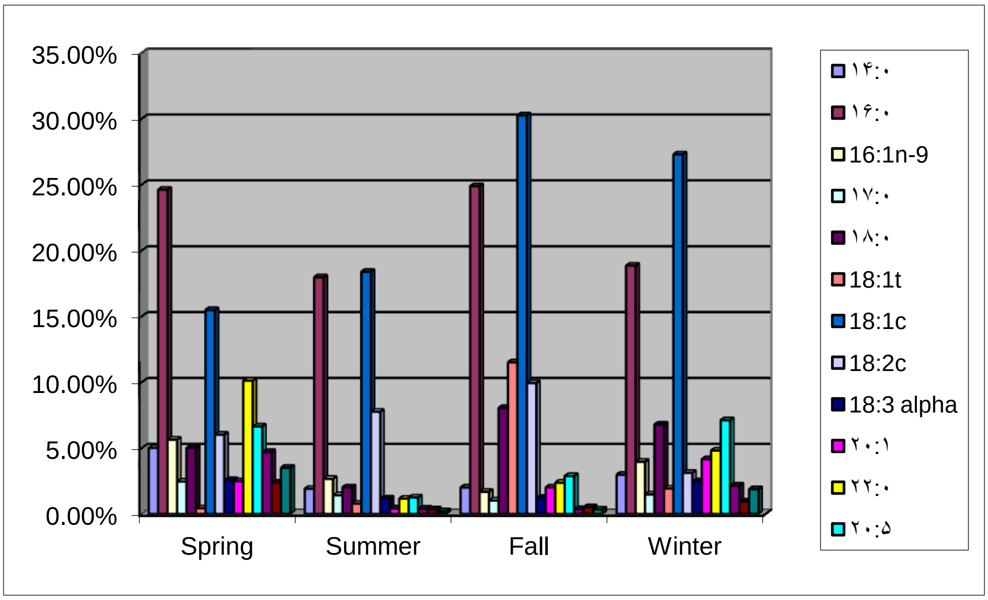

Figure 2- Seasonal variations of fatty acid compositions of *Turbo coronatus*

As shown in Fig 2 gadoleic and eicosapentaenoic acids have their maximum levels in winter and there is a similar trend in palmitic and oleic acids .

In order to obtain any relation between seasonal variations of fatty acids and environmental parameters (temperature, chlorophyll a and salinity), Pearson correlation applied for fatty acid compositions of whole body tissues of *Nerita textilis* and *Turbo coronatus*. Correlation coefficient matrix for twelve fatty acids of *Nerita textilis* and environmental factors showed strong negative correlation (r=-0.982) between temperature and oleic acid, strong correlation (r=0.967) between chlorophyll a and gadoleic acid and strong correlation (r=0.968) between salinity and margaric acid, all with P< 0.05. At the same way correlation coefficient matrix for fifteen fatty acids of *Turbo coronatus* and environmental factors showed correlation with P< 0.65 only between salinity and lignoceric acid with strong negative correlation (r=-0.951) . Due to the Pearson analysis results and significant coefficients for mentioned fatty acids and environmental parameters , univariate regression analysis was applied by considering environmental factors as independent variable and each fatty acid as dependent and gave r^2 values of 0.947 , 0.934 and 0.938 for temperature , chlorophyll a and salinity respectively (table 3 , 4 and 5) which implies strong effects of these parameters on related fatty acids in *Nerita textilis* . The regression analysis for *Turbo coronatus* between lignoceric acid and salinity showed r^2 equal with 0.905 (table 6) and implies again the strong effect of this parameter on lignoceric acid.

According to regression analysis and tables 3-6, fatty acids are predictable by applying regression equation of follows:

$Y=a+b_1x_1+b_2x_2+b_3x_3$ …

Where, Y is the unsaturated coefficient (amount of fatty acid), parameter a is constant and is derived from tables 3-6, b is efficiency coefficient and is calculated in the tables 3-6 either and x will be the measured value for temperature at any given time.

Table3- Regression analysis for temperature and oleic acid in *Nerita textilis*

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	62.922	20.440		3.078	.091
	Temp	-1.433	.726	-.813	-1.975	.187

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.813(a)	.661	.492	5.02192

Table4- Regression analysis for Chlorophyll a and gadoleic acid in *Nerita textilis*

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	4.942	3.672		1.346	.311
	Chl	-6.837	9.160	-.467	-.746	.533

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.467(a)	.218	-.173	1.66693

Table5- Regression analysis for salinity and margaric acid in *Nerita textilis*

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	35.957	9.572		3.757	.064
	Salinity	-.943	.263	-.930	-3.590	.070

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.930(a)	.866	.799	.27459

Table6 - Regression analysis for salinity and lignoceric acid in *Turbo coronatus*

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	53.714	12.079		4.447	.047
	Salinity	-1.445	.331	-.951	-4.360	.049

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.951(a)	.905	.857	.34652

Discussion

The fatty acid components of *Nerita textilis* and *Turbo coronatus* were different and also there was not any similarity between the seasonal variations of common fatty acids in two species.

The major fatty acids in two species were palmitic and oleic acids which coordinate with previous reports (Ackman, 2000; Feuntes *et al.*, 2009) introduce these acids the main fatty acids in mollusks especially in all trophic levels.

In *Nerita textilis* unsaturated fatty acids was most abundant and dominated over saturated ones which coordinate with other reports (Feuntes, 2009 ; Frietes *et al.*, 2002) while in *Turbo coronatus* there was found an opposite result which saturated fatty acids were dominants. These findings could lead to resulting that even in a common habitat and food supply; species of a same class have different dietary, metabolism and behaviors.

The maximum level of unsaturated fatty acids in *Nerita textilis* was observed in winter and its minimum was in summer, and coordinates with Other researches which had reported an inverse relationship between temperature and the amount of poly unsaturated fatty acids in tissue lipids of invertebrates, due to the adaptive regulation of melting point of cellular lipids (Chu & Greaves, 1991; Pazos *et al.*, 1996).

The saturated fatty acids were dominated over unsaturated ones in *Turbo coronatus* and this finding is in the opposite manner of above reports.

Most important environmental factors altering tissue lipid levels of invertebrates are temperature, chlorophyll a and salinity. These, together may be contributed to seasonal variations in fatty acid compositions of *Nerita textilis* and *Turbo coronatus* , According to this purpose Pearson analysis was done for fatty acids of two species in relation to temperature , chlorophyll a and salinity .

In the case of *Nerita textilis* oleic acid showed correlation with temperature and implies the inverse effect of temperature on such unsaturated fatty acids which increase with decreasing temperature and fatty acid denaturizes are responsible for maintaining the appropriate fluidity of membranes under this condition because many invertebrates respond to low temperature by adjusting capacities of enzymes from energy metabolism , restructuring membrane phospholipids and modulating membrane fluidity (LOS & Murata , 1999 ; Crockett & Dougherty, 2000) .

On the other hand, maximum lipid concentrations and high unsaturated fatty acids occur in high phytoplankton biomass which relates to primary production and chlorophyll a (Skerratt & Nichols , 1995), and coordinates with the findings in this research which gadoleic acid had significant correlation with chlorophyll a.

In *Nerita textilis* and *Turbo coronatus* salinity showed a strong correlation with margaric acid and lignoceric acid respectively, and according to other researches fatty acids of many marine organisms are influenced by different levels of salinity (Rao & Dayananda, 2007).

Consequently, it should be emphasized that fatty acid contents of *Nerita textilis* and *Turbo coronatus* are not similar but have a few acids in common and it could be observed that seasonal variations of these common fatty acids are not the same at two gastropods. The major fatty acid in *Nerita textilis* is Palmitic acid and Poly unsaturated fatty acids in *Turbo coronatus* is higher than *Nerita textilis*. Therefore, it could be concluded that *Turbo coronatus* dietary is more higher plants than *Nerita textilis* .

Among ecological factors temperature, chlorophyll a and salinity showed relativity with fatty acids in *Nerita textilis* but only salinity showed correlation with lignoceric acid, in *Turbo coronatus.*

Considering obtained results, generally we could conclude that there is not an absolute existing similarity between fatty acid contents of two species of a same class even in one common habitat and it depends on many factors such as internal metabolism, food dietary and reproduction cycle.

Since little published reports exist on lipids of gastropods of Chabahar bay area, this research would be of value for other researchers that are interested in this field.

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