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Lipid Characterization of Wild Species *Pinctadaradiata* in Southern Tunisia East

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Abstract

The lipid and Fatty Acids composition of the total lipids of the pearl oyster (*Pinctada radiate)*, in different seasons in southern Tunisia east, were analyzedin order to assess and enhance this species.

Total fatty acid content (79.61±6.18–400.21±50.12mg/100g of dry tissue) varied during the year, reaching the highest value in winter in coincidence with the increase or high levels of phospholipids (PE and PC) were found in the polar lipids especially in winter.

The major FA in the TFA in all seasons were 14:0, 16:0, and 18:0 as saturated FA (saturates); 16:1, 18:1 and C20:1 asmonoenoic FA (monoenes);20:4n-6 (arachidonic acid: AA), 20:5n-3 (EPA), and 22:6n-3 (DHA) as PUFA. The major components found in the polar lipids were 16:0 and 18:0 as saturates; 22:3n-3,22:5n-3,AA, EPA, and DHA as PUFA. It is a marine animal, characteristically high levels ofEPA and DHA were found in both the TFAand phospholipids.

P.radiata lipids contain high levels of both n-3 and n-6 PUFA and, more specifically, that EPA and DHA are the characteristic fatty acids in both the TFA and tissuePE and PC of *P. radiate*

Compared to the percentage of (n-6) series fatty acids contained in the TFA of *P.radiata,* the part of the (n-3) series is more important, yielding n-3/n-6 ratios of respectively 3.12, 3.11, 2.13 and 2.15 in winter, spring, summer and autumn. (n-3) and (n-6) fatty acids contained in the flesh present no significant seasonal variations it only has a low rate of n-6 in automn compared to other seasons.

Keywords: Seasonal variation, Total fatty acids (TFA), phosphatidylcholine (PC) phosphatidyl ethanolamine (PE), poly-unsaturated fatty acids (n-3) and(n-6), (*Pinctada radiate)*.

Introduction

Molluscs are one of the most important group of invertebrates **(Joseph, 1982).** In particular, bivalves such as oysters (Ostreidae), are now among the most interesting inhabitants of the seas for both scientific and economic reasons because they have become of increasing importance as food. Although the lipid composition of many marine molluscs is well documented, there is only a limited amount of information on the fatty acid and phospholipids composition of these Mediterranean molluscs especially *P.radiata.*

The pearl oyster, *P.radiata* **(Leach, 1814)**or Rayed pearl oyster **(Fischer *et al*,1987)**is an Indo-Pacific origin sessile benthic species. In Tunisia, only one study on the biological reproduction of this species harvested around the islands of Kerkennah was carried out until today **(Tlig-Zouari, 2009).**

In Tunisia, *P.radiata* hasn't been much exploited; its consumption is limited to Kerkennah and Djerba and is often discharged into the sea in a fishing accident. Moreover, it does not produce pearls and even when they are present, they are of poor quality and very friable **(Seurat, 1929).**This species is abundant in the Gulf of Gabes particularly in Mahares, but it becomes scarce with sporadic repartition in the Gulf of Tunis **(Tlig-Zouari, 2009).**

In our diets, fatty acids,especially phospholipids, which are integral parts of biomembranes and usually are rich in polyunsaturated fatty acids **(Paoletti and Kritchevsky,1989).**Two main fields of interest in the research on the

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essentiality of PUFA are membrane integrity and the formation of prostaglandins and eicosanoids from n-3 and n-6 PUFA **(Kinsella, 1981).** These are of major importance in membrane fluidity and influence the permeability of membranes and the behavior of membrane-boundenzymes and receptors **(Paoletti and Kritchevsky, 1989).** Prostaglandins are involved in the modulation of a large and diverse number of vital physiological functions **(Kinsella, 1981).**
Arachidonicacid (C20:4n-6) is represented in smaller quantities and is more characteristic of urchins and starfish **(Isay and Busarova, 1984).**Eicosapentaenoic acid (C20:5, n-3) is a characteristic of invertebratesdietary supplements or dietary n-3 PUFA, such as DHA (22:6 n-3), may have beneficial effects on cardiovascular diseases, certain forms of cancer and aging **(Shahidi&Miraliakbari, 2004**).

According to literature data the content of C22:6 may differ from year to year, from season to season and may depend on the nutrition of organisms. The composition of the polyunsaturatedfatty acids of marine organisms is generally influenced by many factors **(Patton, 1975).**

The object of the present work is the quantitative and qualitative determination of the total fatty acids andthe most important polar lipids PC and PE which depending to the composition of PUFA of the shell of *P.radiata* focusing on the fatty acid composition. We decided to study seasonal variations to figure out the richest season in PC and PE and especially EPA and DHA to consume this species.

Material and Methods

This species is collected using the benthic trawl of oceanographic vessel HANNIBAL.

Sampling was carried out in February 2011 to November 2012 at the region of Mahares, as a pilot site away from harbor areas since it provides oysters with relatively thick pearly shells **(Fig.1,tab I).**

Animals

The fleshes were removed from the collected specimens and were fixed in boiling water to complexly inactivate enzymatic activity, especially phospholipases**(Shewfelt, 1981)**. Samples along with the fixing liquid were stored at -28°C.

Total lipid extraction

Lipids were extracted according to the **Folch, Lees and Sloane- Stanley (1957)** method with the solvent mixture chloroform–methanol (2:1, v/v) containing 0.01% butylatedhydroxytoluene (BHT) as an antioxidant **(Christie, 1982).**

Polar lipid extraction

The crude total lipids (TL) were separated into classes on plates of silica gel **(Silica gel 20 * 20, layer; 0.25 mm silica gel, Merck and Co. Ltd., Darmstadt, Germany).**

Fatty acid analysis

After evaporation to dryness, lipid extracts were trans-esterified according to the **Cecchi, Basini, and Castano (1985)** method. Methyl nonadecanoate C19:0 (Sigma) was added as internal standard. Separation of FAMEs was carried out on a HP 6890 gas chromatograph with a split/splitless injector equipped with a flame ionisation detector at 275 °C, and a 30 m HP Innowax capillary column with an internal diameter of 250 µm and a 0.25 µm film thickness. Injector temperature was held at 250 °C. The oven was programmed to rise from 50 to 180 °C at a rate of 4 °C/min, from 180 to 220 °C at 1.33 °C/min and to stabilize at 220 °C for 7 min. Carrier gas was nitrogen. Identification of FAMEs was based on the comparison of their retention times with those of a mixture of methyl esters (SUPELCO PUFA-3). Fatty acid peaks were integrated and analysed using HP chemstation software.

Identification and quantification of fatty acids

The different fatty acids in *P.radiata* were obtained by comparing the retention times of the fatty acids under study and those of mixture of methyl esters SUPELCO (PUFA-3).

The quantification of the fatty acids is based on an internal standard not present in our samples, methylnonadecanoate or C19:0 (Sigma).

Statistical analysis

Data were analyzed using the software Statistica Version 6.0 to assess significant differences between means according to the one way analysis of variance method (ANOVA). For this, the Duncan test was applied and differences were considered significant when $p < 0.05$.

Results

Biological data on the *P. radiate* samples are listed in Table 1. The sizes and weights of samples of Februaryand augustwere markedly greater than those of samples of May and November, showing that the *P. radiate* grew during the growing season.

The TL content and lipid classes are shown in Table II, III and IV. The TL content was 79.61-400.21mg/100g in all season.In addition; the lipid contents were slightly higher (334.28–400.21mg/100g) during the spawning season (February and May) ($p<0.05$) than in the growing

season (79.61–187.06mg/100g), November and August.

The total lipid content of *P.radiata* samples is shown in Table II, accompanied by the lipid classes(Tab III and IV). The fatty acids derived from seafood oils are of three principal types: saturated, monounsaturated and polyunsaturated. TheSFA content in the flesh ranged from37.93to 214.06mg/100g of total FA in all four seasons, while the PUFA ranged from34.77 to149.82mg/100g. Compared with the very low levels (6.90 to36.32mg/100g) of MUFA.The lipid contents of the shell of *P. radiate* were markedly high in winter ($p<0.05$).

The saturated fatty acids predominated in the winter (214.06 mg/100g), the lowest levels were found at the end of the autumn and early summer. The polyunsaturated fatty acids underwent variations (34.77 to149.82mg/100g) and showed a similar seasonal pattern to the total levels of n-3levels of n-6were low in winter, then increased significantly between Spring and Autumn season($p<0.05$). Seasonal changes in the monounsaturated fatty acids were less important (6.90 to36.32mg/100g).

The most important saturated fatty acids: 14:0 (5.79–6.06%), 16:0 (25.76–34%) and 18:0 (8.71–12.65%) and monounsaturated fatty acids: 16:1(3.59–4.56%), 18:1n-3 (2.32– 4.01%) and 20:1 (0.13–1.4%) showed similar seasonal profiles.

The most abundant polyunsaturated fatty acids (PUFA) were: 20:5(n-3) (4.18–5.78%), 22:6(n-3) (13.94–16.04%) and 18:4(n-3) (0.25–1.01%). The seasonalprofiles of all these were similar and parallel tothat of the PUFA. The (n-6) PUFA containedhigher percentages of 20:4(n-6) than of 18:2(n-6) and the seasonal variations of the latter followedthose of the (n-3) PUFA quite closely. The percentagelevels of (n-3) PUFA were significantlyhigher than those of (n-6) PUFA ($p<0.05$) (Tab.II).We noticed also the presence of Non-interrupted methylene dienoic (NMID) (C22: 2): C22: 2i (0.40% and 1.59) and C22: 2j (0.07% and 0, 30%) in small quantities.

The highest levels of PUFA of PC were registered between the middle of the summer (August) and the endof the winter (February) ($p<0.05$), then decreased slightly and remained low during the autumn and spring (Tab.III).

The PUFA of PC showed a significant relationship with the seasonal changes n-3 series of PC decreasing from 25.03at the beginning of the study (February) to11.25mg/100g in May, remaining low until August1.74 mg/100g, and finally increasing up until November.

The seasonal variations in FA of PE are shown in Table IV. The lowest values of PUFA were registered in spring– autumn (1.27–0.62mg/100g of the total FA) and the highest in winter (maximum in February22.25mg/100g of the total FA) ($p<0.05$). The percentage of SFA ranged between5.64 in winter and0.69 mg/100gin summer.

The seasonal variations in the n-3 series of PC and PE were very similar to those of the TFA especially between them.

On the one hand, EPA and DHA increased in winter in PC and PE ($p<0.05$). On the other hand, a positive relationship existed between total FA and PC and PEphospholipids; this correlation was stronger when the data were expressed as mg/100g of shell of total lipids.

The lipid classes in winter differed from those in otherSeasons; in particular, the levels of PUFA in the sample were highest (32.10±9 mg/100g forPC and 22.25±6mg/100gfor PE). This result may also have been caused by a less favorable condition such as the lower seawater temperature; the high levels of PCand PEmight be viewed as the result of important levels of TFA.

The seasonal composition of FA of Total Fatty Acids and (EPA+DHA) of Total Fatty Acids, PC and PE of *P.radiata* are listed in Table V.The contents of TFA of PC is marquely higher than those of the TFA on the one hand and those of PE on the other hand also in the composition of (EPA+DHA) in all seasons except Autumn but these contents decreased marquedly in summer.

Discussion

The fatty acids derived from seafood oils are of three principal types: saturated, monounsaturated, and polyunsaturated **(O'Keefe Ackman, 1987)**

All of the invertebrates studied containedlarge amounts of PUFA. (The average of four seasons 40.63±3.05% of total fatty acids) in *P. radiata*. Some authors reported that the proportions of saturated, monounsaturated and polyunsaturated FA in species of bivalve molluscs from the Mediterranean sea **(Dridi*et al.*, 2008)** were in the range of 23±26, 12±15% and 57±65, of total fatty acids, respectively, and in species of gasteropod molluscs from Pacific ocean and east China Seathey ranged from 26.7 to 34.1, 10to 35.1 and 27.6 to 64.6% of total fatty acids, respectively **(Saito, 2014).**

These results are in agreement with those in the bivalve studied (47.11 to54.47,8.41 to 9.64 and 36.66 to 44.46% of total fatty acids, respectively).

In our Mediterranean species the content of polyunsaturated fatty acids is high, which is in

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agreement with the literature data **(Dridi *et al.*, 2008; Telahigue *et al.*,2010)**.

The dominate fatty acids identified in the shell of this pearl oysterwere: 14:0, 16:0, 18:0, 16:1, 18:1, 18:3n-3, 20:4n-3, 20:5n-3 (EPA), 22:5n-3 (DPA) and 22:6n-3(DHA). These fatty acids contribute approximately 60-75% of the total FA.All seasons contained high proportions of palmitic acid C16:0 (25.76 to 34% of total fatty acids) and stearic acid C18:0 (8.71-12.65% of total fatty acids).

The most characteristic polyunsaturated fatty acids of *P.radiata* were found to be docosahexaenoic acid (C22:6 n-3) and eicosa-pentaenoic acid (C20:5 n-3), which ranged between 13.94 and 16.04 and 4.18 and 5.78% of total fatty acids. In the literature it is also reported that DHA and EPA are the most characteristic acids for molluscs, ranging between 20 and 36, and 8.3 and 17.3% of total fatty acids **(Culkin and Morris, 1970; Gibson, 1983).** Arachidonic acid (C20:4 n-6), was found in proportions that ranged from 3.69 to 7.44% of total fatty acids. **Daria *et al.*, (2012)** also found arachidonic acid to be 5.10% of total fatty acids in *Mytilus galloprovincialis* from Mali Ston Bay and in Ostreaedulis from the same region it ranged from 0.30 to 4.65% of total fatty acids.

Also, we noticed the presence of Non-interrupted methylene dienoic (NMID) (C22:2): C22:2i (from 0.4 to1.59 mg/100g) and C22:2j (from 0.07 to 0.3mg/100g). According to several authors, these fatty acids (C22:2i and C22:2j) are involved in the mechanisms of fluidity, integrity and structure of the membrane **(Gilles, 2009).** The presence of fatty acids C22:2i and C22:2j bivalve confers protection against membrane alterations due to changes in the physicochemical environment. **Gilles (2009)** reported the bivalve molluscs ability to synthesize polyunsaturated fatty acids called indeterminate C22:2i and C22:2j. In this study, the percentages of these fatty acids were recorded in the flesh in low quantities.

Among the various phospholipids that may occur in the marine organisms, lecithin PC is the main phospholipids in marine invertebrate and vertebrates, while PE is the second most common class. Some sponges, soft corals, and mollusks may contain more PE than PC.

The five FA were also the major components in the PC: 16:0 (27.54–39.29%) and 18:0 (5.97–7.98%) as saturates; AA (3.21–4.37%) as n-6 PUFA; and EPA (2.75–6.98%) and DHA (10.45–22.93%) as n-3 PUFA. Significant amounts of 10 FA—17:0, 22:0 as saturates; 18:1n-9, 18:1n-7, and 20:1n-9 as monoenes; 22:2i-2j, as NMID; 22:3n-3and 22:5n-3 (DPA: docosapentaenoic acid) as n-3 PUFA and20:2n-6;18: 2n-6 as n-6 PUFA—were found in the PC.

Although the detailed FA levels of the PE differed slightly from those of the PC, the profile of these major components of the PE were close to those of the PC. In particular, the total amounts of PUFA in both PE and PC (0.62–22.25mg/100g for PE and 2.73–32.1 mg/100g for PC) were high under all fourseasons; therefore, the PE and PC of the *P. radiate* were rich sources of PUFA, similar to those in other marine animals **(Takama *et al*; 1994; Medina *et al.*, 1995**). The major fatty acid composition of the shell of *P. radiate* are presented in Tables III and IV of PC and PE The fatty acid composition in PE included noticeable levels of 18:0 and 20:1 (Tab.IV), similar to those in other mollusk species **(Saito, 2004; Saito and Hashimoto, 2010; Sargent, 1989**). This finding is the general characteristic for mollusks containing like *T. cornutus.*

The high levels of total PUFA in the Polar Lipids (PL) especially in winter (important amount) suggest the accumulation of PUFA in the tissue, because bivalves may not be able to biosynthesize PUFA such as AA and DHA **(Joseph, 1989; Cook, 1991).** In particular, the high levels of AA, EPA, and DHA might be the result of a concentration of these PUFA in the tissue, because high levels of PUFA were consistently found in the PL, compared with those in TFA.

Moreover, significant levels of n-6 PUFA, such as AA, were found in the PL, compared with the high levels found in the TFAespecially in winter. Similarly, the level of AA in the PL increased slightly, compared with that in theTFA. As for n-3 PUFA, the level of DHA increased markedly in both the PE and PC, and DPAincreased slightly in the PE, whereas the shorterchain unsaturates 18:4n-3 and EPA decreased in the PC and PE compared with those in the TFA. The species may selectively concentrate longer and more highly UFA in its tissues as membrane lipids.The contents of TFA and of (EPA+DHA) of PC is marquedly higher than those of the TFA and PE in all seasons but decreased marquedly in summer, it is explain by the spawning season.

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The study of the reproductive cycle reveals that a sexual activity of Pintadine throughout the year **(Zouari, 1994).**The fertilization is external, such a mode of reproduction causes a huge loss of gametes, offset by the significant fertility individuals especially females **(Herdman & Hornell, 1906),**but spawning occurs mainly during the summer. Gonad maturation is closely related to temperature **(Zouari, 1994).** During the spawning season, the DHA content in PC fluctuated between 10.45 and 22.93% of TFA (autumnand winter). This amount may be influenced by environmental and seasonal differences in the prey phytoplankton lipids, because the total PUFA levels (NMID, n-4, n-6, and n-3 PUFA: 45.07% of TFA for specimens in winter, and 33.5% for those of autumn) in the spawning season did not differ much from each other. Otherwise, in the spawning season, the fluctuation of its FA composition might be influenced by maturation, because differences in the concentrations of specific chemical components in the tissues were often found between before and after spawning

For instance, the levels of long-chain 22:5n-3 and 22:6n-3 increased markedly, while there were decreases of 16:0, 18:0 and 22:0 in the PE, compared with high levels of the shorter-chain and less unsaturated FA in the TFA. This result suggests that these long chain might originate from 18:0 and 22:0 significantly accumulation of n-3PUFA in the lipids of *P. radiata*.

In general, DHA is the dominant PUFA in both the PE and PC of almost all higher trophic-level marine animals after accumulation in the food chain (**Takama, 1994; Medina, 1995; Saito, 1999).** Although the high levels of n-3 PUFA in *P. radiata* PL were also similar to the results reported for other marine animals (**Takama, 1994)**, fluctuations of the respective PUFA were found in the polar lipids. For example, the DHA in PE fluctuated from 6.52 to 33.63%, and that in PC fluctuated from 10.45 to 22.93%;

FAcomposition of marine organisms is characterized by significant amounts of 20 and 22 carbon chain length, highly-unsaturated, n-3 fatty acids **(Stansby, 1982).**Another study indicates that the composition of fatty acids in oysters will have seasonal variabliy, which is related to seawater temperature and food supply.

According to **Seurat (1929a),** the acclimatization of the small Mediterranean pintadine present a major scientific interest because of its significant expansion in time as short interval. Furthermore, this species has a very high resistance. Indeed, as mentioned it can stay dry at low tide for a few hours at very high temperatures.In this case seasonal FA composition can possibly related to food supply and reproduction.

The fatty acids showing the greatest seasonal changes belong to the n-3 series **(Abad *et al*., 1995)** differing from of marine gastropod as *Turbo cornutus wich* accumulates n-6 PUFA as a possible substitute for n-3 PUFA in order to maintain cell membrane fluidity **(Saito, 2014)** and according to **Tocher (2003, 2010),** the essentiality of n-3 PUFA is for marine fishes.Some research suggests that n-3 fatty acids help in diminishing the undesirable effects of inflammatory diseases, have beneficial effects on stroke, may reduce the incidence of breast cancer, and may help alleviate certain skin diseases **(Stansby, 1982).**

No seasonal difference of the n-3/n-6 PUFA ratio of *P. radiata* in total lipids was found to be 2.13-3.12. Fatty acid profiles of TFA and polar lipids (PC and PE) of the studied oyster presented a differentiation more in the proportions than in the variety of fatty acids

This finding characterizes the high nutritional value of this species. In fact, this ratio ranged from 4.28 to 10.81 in (clams) bivalve mollusk the *Chamelea Gallina*from the central Adriatic Sea**(Orban, 2006).** Besides **Fuentes *et al*., (2009)** registered n-6/n-3 values varied between (0.09and0.13) in *Mytilusgallo provincialis* from Spanish coast. A healthy balance between n-3 and n-6 PUFA by consumption of seafood is recommended by current dietary guidelines **(Orban, 2006).**

Relationship between polar lipids (PC and PE) and the concentration of total fatty acids in the lipids of *P. radiate* explain the high level of PUFA.The turn-over of PLs is likely a relatively-rapid phenomenon in bivalves, in light of rapid adaptation of cell membranes during temperature changes **(Farrias *et al*., 2003).** All marine animals may have a tendency to accumulate PUFA in their tissues, and the difference in n-6 and n-3 PUFA levels among bivalve species may be caused only by their feeding habits.

Conclusion

Like most marine animals *P. radiate* have high levels of PUFA especiallythe total lipids and principal lipid classes PC and PE which are the principal lipid class and appear to have structural role for the fluidity of the cell membranes. The seasonal variations in lipid content were basically due to changes in accumulation and utilization. Many factors impact the fatty acid composition, such as diet,

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geographic locations of catch and seasons of the year, which may be related to water temperature (**Pennarun *et al.*, 2003).**

The comparatively high lipid levels in the spawning season suggest that the lipids may play some role in maturation **(Awaji and Suzuki, 1995**) similar to that in finfishes (**Henderson *et al.*, 1984).** Otherwise, the higher lipid contents inwinter and spring suggest that the warm seawater might have promoted accumulation of lipids related to a high metabolic activity in this season.

The lowest lipid content, seen in autumn, suggests that the most unfavorable condition, i.e., the low temperature in this season, disturbed the normal lipid metabolism.The fatty acid composition profile in the shell of *P. radiate* was influenced by the temperature, food availability, reproduction. The influence of seasons on the TFA profile of the flesh of *P. radiate* is clearly shown by its positive correlation with the composition of Fatty acids of PC and PE and correlation with PUFA levels especially in winter and reveals feeding selectivity for specific classthroughout the year.

The present study confirmed that the *P. radiata* lipids contain high levels of both n-3 and n-6 PUFA and, more specifically, that EPA and DHA are the characteristic fatty acids in both the TFA and tissuePE and PC of *P. radiate*

In this work, we have presented the first data about the fatty acid composition of the Tunisian shell of *P. radiata*. With a 100-gram of shell serving providing 0.22 grams of EPA, and DHA, combined. This amount is more per serving than contained by comparable servings of crab, lobster, mussels, clams or fish like snapper or catfish and 300g of shell of *P. radiata* (about 6 medium oysters) have 750mg per day of Omega 3 Fatty Acids.

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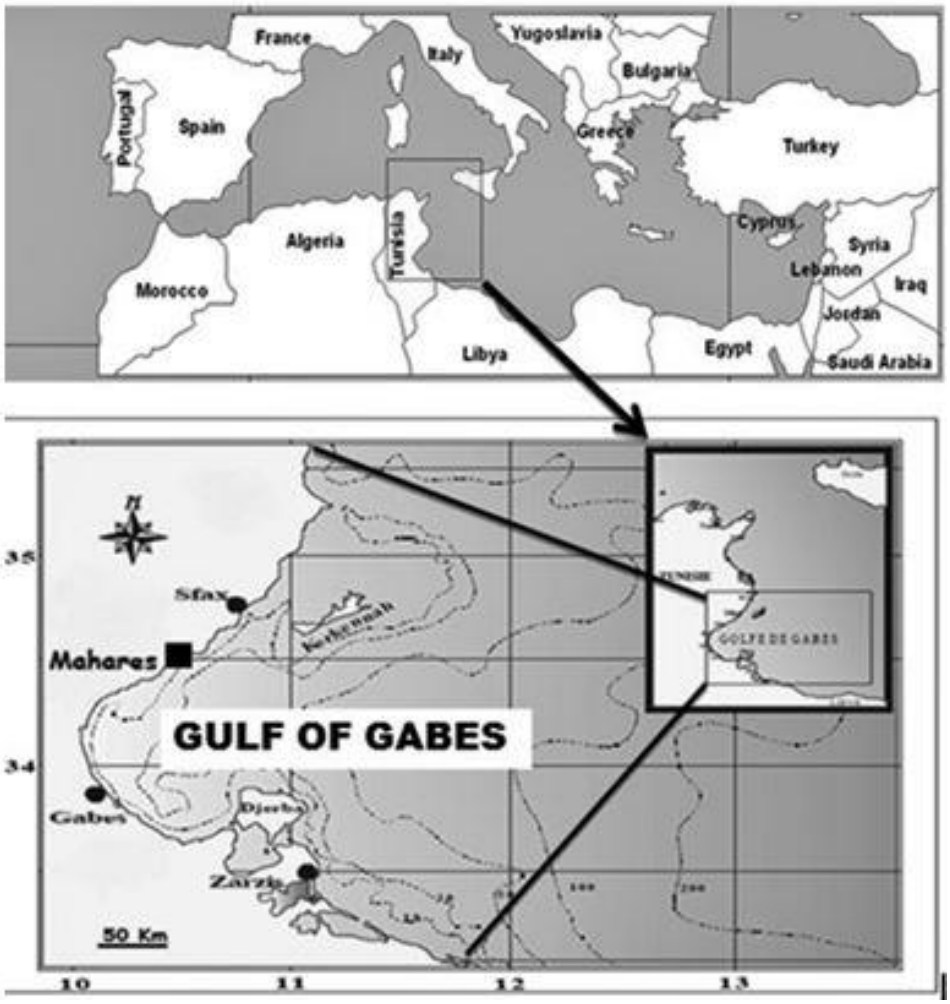

Figure 1. Geographical location of the sampling site Mahares at the Golf of Gabes (■).

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Table.I. Cultivation Locality and Biological Data of the Pearl Oyster *P. radiata*in Mahares.Results are expressed as mean ± SE (*n* =6)

Locality		Depth(m)		Replicate Animals (n)	Salinity,‰	Temperaures (°C)	Date	Lengh (cm)	Widh (cm)	Weight (g)	Total Fatty Acids Mg/100g
beginning	**End**	**beginning**	**End**								
34°25',818N 10°58',91E	34°26',661N 11°03',080 E	19.6	21.2	6	36,6-39,7	16-18	February 26,2011	8.7±0.57	7.83±0.42	106.25±7.26	400.21±50.12
						19-20	May 15,2012	8.57±0.29	7.55±0.28	88.37±7.84	334.28±76.8
						22-27	August 26,2012	5.65±0.27	4.42±0.22	38.51±4.91	187.06±8.97
						22-25	November 19,2012	6.4±0.12	5.48±0.11	54.76±3.09	79.61±6.18

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Table.II. Seasonal variations in fatty acid composition of TFA of *P.radiata*(mean ± SE; *n* = 6).
a,b,c – values in rows with different letter indexes differ significantly P≤ 0.05.

%	Winter	Spring	Summer	Automne
C14:0	6.06±0.46^{a}	6.84±1.18^{a}	6.53±0.69^{a}	5.79±1,1^{a}
C15:0	0.27±0.11^{b}	0.53±0.04^{b}	1,24±0.04^{a}	1.16±0.11^{a}
C16:0	34.00±1.23^{a}	28.50±3.02^{ab}	31.91±1.62^{ab}	25.76±1.47^{b}
C17:0	0.03±0.01^{b}	0.50±0.04^{b}	0.47±0.04^{b}	1.52±0.41^{a}
C18:0	12.65±1.74^{a}	8.71±0.78^{a}	10.48±0.49^{a}	11.19±0.53^{a}
C22:0	1.56±0.16^{a}	2.24±0.58^{a}	1.45±0.12^{a}	1.67±0.11^{a}
C14:1	0.79±0.05^{a}	0.10±0.09^{a}	1.00±0.11^{a}	0.84±0.06^{a}
C15:1	0.28±0.06^{b}	0.69±0.17^{ab}	0.88±0.12^{a}	0.58±0.1^{ab}
C16:1	3.59±0.78^{a}	3.82±0.5^{a}	4.56±0.41^{a}	3.68±0.68^{a}
C18:1n-7	trace	trace	trace	trace
C18:1n-9	2.80±0.24^{b}	4.01±0.42^{a}	2.32±0.16^{b}	3.17±0.29^{ab}
C20:1n-9	1.40±0.18^{a}	0.81±0.55^{ab}	0.86±0.09^{ab}	0.13±0.05^{b}
C22 :1n-9	trace	trace	trace	trace
C18:2n-6	1.88±0.16^{a}	2.39±0.22^{a}	1.95±0.17^{a}	1.57±0.3^{a}
C20:2n-6	1.18±0.4^{a}	7.92±7.10^{a}	4.77±4.22^{a}	4.88±3.15^{a}
C20:4n-6	4.88±0.5^{ab}	3.69±0.51^{b}	5.60±1.14^{ab}	7.44±0.57^{a}
C18:3n-3	0.29±0.12^{b}	1.50±0.51^{a}	1.04±0.06^{ab}	0.9±0.09^{ab}
C18:4n-3	1.01±0.09^{a}	0.59±0.27^{ab}	0.58±0.10^{ab}	0.25±0.13^{b}
C20:4n-3	0.32±0.04^{a}	0.25±0.09^{a}	0.24±0.15^{a}	0.24±0.06^{a}
C20:5n-3	5.78±0.71^{a}	4.18±0.38^{a}	4.19±0.43^{a}	4.18±0.42^{a}
C22 :3n-3	trace	trace	trace	trace
C22:5n-3	0.92±0.1^{a}	2.18±0.51^{a}	1.30±0.1^{a}	5.45±3.32^{a}
C22:6n-3	16.04±0.82^{a}	15.87±2.85^{a}	14.37±0.9^{a}	13.94±0.75^{a}
C16:2n-4	2.49±0.12^{a}	1.92±0.15^{a}	2.14±0.11^{a}	2.77±0.61^{a}
C16:3n-4	0.53±0.03^{a}	0.29±0.06^{b}	0.25±0.06^{b}	0.65±0.05^{a}
C18:3n-4	0.39±0.1^{b}	1.35±0.24^{a}	0.49±0.14^{b}	0.31±0.14^{b}
C22:2i	0.40±0.19^{b}	0.72±0.32^{ab}	1.16±0.08^{ab}	1.59±0.45^{a}
C22:2j	0.30±0.15^{a}	0.27±0.13^{a}	0.07±0.01^{a}	0.24±0.04^{a}
FA (mg/100g)				
SFA	214.06±21.23^{a}	168.37±50.15^{ab}	97.93±7.04^{bc}	37.93±4.55^{c}
MUFA	36.32±7.33^{a}	32.71±9.82^{a}	18.18±1.60^{ab}	6.90±1.26^{b}
PUFA	149.82±23.07^{a}	133.18±22.82^{a}	70.95±2.51^{b}	34.77±2.19^{b}
UFA	186.14±29.4^{a}	165.90±29.66^{a}	89.13±3.64^{b}	41.67±2.43^{b}
n-3	100.21±15.35^{a}	79.63±18.37^{a}	40.87±3.31^{b}	19.64±2.18^{b}
n-6	32.88±5.78^{a}	38.67±15.83^{a}	22.38±4.53^{a}	10.68±2.08^{a}
EPA+DHA	89.75±13.75^{a}	64.97±15.66^{ab}	34.87±3.03^{bc}	14.39±1.28^{c}
n-3/n-6	3.12^{a}	3.11^{a}	2.13^{a}	2.15^{a}

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Table.III. Seasonal variations in fatty acid composition of PC of *P. radiata* (mean ± SE; $n = 6$).
a,b,c.d – values in rows with different letter indexes differ significantly $P \leq 0.05$.

%	Winter	Spring	Summer	Autumn
C14:0	1.66±0.08^{c}	3.13±0.18^{b}	4.12±0.65^{ab}	4.66±0.21^{a}
C15:0	0.78±0.04^{b}	1.27±0.09^{b}	1.96±0.7^{b}	3.12±0.21^{a}
C16:0	33.59±1.54^{ab}	39.29±1.64^{a}	30.27±4.49^{ab}	27.54±1.38^{b}
C17:0	2.32±0.42^{b}	0.93±0.13^{b}	4.44±0.87^{a}	2.41±0.12^{b}
C18:0	6.36±0.57^{a}	7.98±0.65^{a}	6.18±0.9^{a}	5.97±0.37^{a}
C22:0	0.53±0.16^{a}	0.57±0.04^{a}	0.45±0.07^{a}	0.71±0.13^{a}
C14:1	0.13±0.1^{c}	0.33±0.04^{bc}	0.89±0.27^{b}	4.13±0.31^{a}
C15:1	0.09±0.01^{b}	0.46±0.1^{b}	1.06±0.54^{b}	2.44±0.19^{a}
C16:1	1.75±0.05^{d}	4.12±0.44^{c}	5.92±0.47^{b}	7.88±0.53^{a}
C18:1n-9	3.11±0.12^{a}	3.48±0.17^{a}	2.25±0.26^{b}	1.95±0.23^{b}
C18:1n-7	2.07±0.13^{a}	1.90±0.19^{a}	2.07±0.39^{a}	1.97±0.19^{a}
C20:1n-9	1.31±0.07^{a}	0.07±0.01^{b}	0.07±0.01^{b}	1.46±0.39^{a}
C22:1n-9	1.16±0.09^{a}	0.53±0.04^{a}	1.20±0.47^{a}	2.19±1.01^{a}
C18:2n-6	2.33±0.12^{a}	2.18±0.11^{a}	1.84±0.22^{ab}	1.45±0.12^{b}
C20:2n-6	1.73±0.29^{a}	2.84±0.16^{a}	2.70±0.53^{a}	2.10±0.18^{a}
C20:4n-6	4.17±0.14^{a}	4.37±0.36^{a}	3.21±0.6^{a}	3.82±0.22^{a}
C18:3n-3	1.43±0.04^{a}	1.03±0.16^{a}	1.18±0.25^{a}	0.82±0.07^{a}
C18:4n-3	0.19±0.03^{v}	0.95±0.13^{a}	0.35±0.07^{bc}	0.51±0.05^{b}
C20:4n-3	0.41±0.05^{a}	0.19±0.02^{a}	0.76±0.48^{a}	0.14±0.03^{a}
C20:5n-3	6.98±0.7^{a}	3.52±0.2^{b}	2.75±0.35^{b}	3.38±0.35^{b}
C22: 3n-3	1.77±0.1^{a}	0.18±0.03^{a}	2.97±1.42^{a}	1.94±0.85^{a}
C22:5n-3	1.21±0.1^{a}	0.79±0.03^{a}	2.93±1.92^{a}	2.77±1.11^{a}
C22:6n-3	22.93±0.9^{a}	14.25±0.56^{b}	14.47±0.92^{b}	10.45±0.83^{c}
C16:2n-4	0.75±0.38^{a}	0.47±0.13^{a}	2.70±1.74^{a}	1.29±0.19^{a}
C16:3n-4	0.19±0.03^{d}	3.45±0.21^{a}	2.49±0.45^{b}	1.03±0.13^{c}
C18:3n-4	0.06±0.01^{a}	0.75±0.25^{a}	0.35±0.16^{a}	0.92±0.54^{a}
C22:2i	0.73±0.18^{b}	0.04±0.007^{b}	0.15±0.04^{b}	1.37±0.54^{a}
C22:2j	0.13±0.11^{a}	0.81±0.33^{a}	0.15±0.04^{a}	1.45±0.75^{a}
FA (mg/100g)				
SFA	30.91±7.75^{a}	28.52±8.38^{a}	3.30±0.65^{b}	12.36±1.73^{ab}
MUFA	6.69±1.72^{a}	5.32±1.20^{a}	0.92±0.14^{b}	6.09±0.9^{a}
PUFA	32.10±9^{a}	18.60±5.04^{ab}	2.73±0.49^{b}	9.22±1.25^{b}
UFA	38.80±10.72^{a}	23.93±6.19^{ab}	3.66±0.63^{b}	15.32±1.9^{b}
n-3	25.03±7.11^{a}	11.25±3.38^{b}	1.74±0.29^{b}	5.40±0.69^{b}
n-6	5.97±1.65^{a}	4.66±1.09^{ab}	0.58±0.16^{c}	2.05±0.28^{bc}
EPA+DHA	21.43±6.09^{a}	9.43±2.75^{b}	1.26±0.29^{b}	3.71±0.34^{b}

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Table.IV. Seasonal variations in fatty acid compositionof PE of *P. radiate* (mean ± SE; *n* = 6).
a,b,c.d – values in rows with different letter indexes differ significantly P≤ 0.05.

	Winter	Spring	Summer	Autumn
C14:0	1.45±0.18^{b}	8.52±1.91^{a}	10.43±2.19^{a}	10.98±2.33^{a}
C15:0	0.44±0.02^{b}	1.56±0.09^{b}	1.25±0.12^{b}	3.88±0.77^{a}
C16:0	4.18±0.44^{d}	24.56±1.31^{a}	17.20±0.67^{b}	11.08±1.34^{c}
C17:0	1.14±0.07^{b}	1.12±0.12^{b}	1.17±0.12^{b}	1.83±0.27^{a}
C18:0	10.36±0.94^{ab}	12.03±0.69^{a}	8.37±0.62^{b}	5.50±1^{c}
C22:0	0.24±0.03^{b}	0.38±0.27^{b}	2.11±0.35^{a}	2.29±0.65^{a}
C14:1	0.43±0.03^{b}	1.40±0.1^{b}	1.81±0.19^{b}	6.12±1.43^{a}
C15:1	0.12±0.004^{b}	2.79±0.71^{a}	3.08±0.76^{a}	3.62±1.13^{a}
C16:1	4.19±0.43^{b}	13.59±1^{a}	9.79±0.8^{a}	10.15±2.15^{a}
C18:1n-9	0.89±0.08^{d}	3.39±0.33^{b}	4.59±0.39^{a}	2.08±0.38^{c}
C18:1n-7	0.45±0.03^{b}	1.01±0.18^{a}	1.01±0.09^{a}	0.83±0.11^{a}
C20:1n-9	0.08±0.01^{b}	0.38±0.07^{b}	0.25±0.01^{b}	3.12±1.18^{a}
C22:1n-9	8.72±0.86^{a}	2.06±0.42^{b}	1.17±0.19^{b}	1.46±0.47^{b}
C18:2n-6	1.21±0.13^{ab}	1.25±0.28^{ab}	1.83±0.15^{a}	0.82±0.23^{b}
C20:2n-6	1.76±0.08^{a}	1.11±0.81^{a}	3.30±1.48^{a}	0.65±0.56^{a}
C20:4n-6	8.00±0.33^{a}	0.26±0.07^{b}	1.43±0.35^{b}	1.46±0.69^{b}
C18:3n-3	2.40±0.37^{ab}	1.06±0.39^{b}	2.73±0.49^{a}	1.69±0.4^{ab}
C18:4n-3	0.09±0.01^{a}	0.60±0.5^{a}	0.41±0.1^{a}	1.00±0.6^{a}
C20:4n-3	0.33±0.04^{b}	0.26±0.12^{b}	0.52±0.09^{b}	2.60±1.03^{a}
C20:5n-3	12.37±0.94^{a}	1.83±0.49^{b}	1.50±0.37^{b}	2.25±0.68^{b}
C22 :3n-3	trace	trace	trace	trace
C22:5n-3	2.92±0.15^{b}	1.88±0.52^{b}	2.02±0.33^{b}	6.21±1.95^{a}
C22:6n-3	33.63±0.39^{a}	8.45±1.23^{b}	6.52±0.55^{b}	9.97±1.6^{b}
C16:2n-4	0.66±0.04^{b}	3.83±1.41^{b}	13.36±2.17^{a}	0.99±0.17^{b}
C16:3n-4	0.36±0.03^{a}	0.94±0.26^{a}	0.62±0.26^{a}	0.43±0.11^{a}
C18:3n-4	0.13±0.02^{a}	4.09±1.28^{a}	0.92±0.25^{a}	4.08±2.34^{a}
C22:2i	1.20±0.68^{a}	1.00±0.45^{a}	1.76±0.35^{a}	3.44±1.62^{a}
C22:2j	2.12±0.66^{a}	0.53±0.12^{a}	0.71±0.21^{a}	1.35±0.5^{a}
FA (mg/100g)				
SFA	5.64±1.57^{a}	2.54±0.86^{ab}	0.69±0.14^{b}	5.7±1.52^{a}
MUFA	4.84±1.21^{a}	1.22±0.34^{b}	0.36±0.08^{b}	4.22±0.95^{b}
PUFA	22.25±6^{a}	1.27±0.3^{b}	0.62±0.1^{b}	5.98±1.27^{b}
UFA	27.10±7.08^{a}	2.49±0.63^{b}	0.99±0.18^{b}	10.21±1.82^{b}
n-3	17.17±4.74^{a}	0.7±0.21^{b}	0.23±0.04^{b}	3.75±0.7^{b}
n-6	3.61±0.91^{a}	0.12±0.04^{b}	0.1±0.02^{b}	0.48±0.2^{b}
EPA+DHA	15.29±4.31^{a}	0.52±0.16^{b}	0.13±0.03^{b}	1.81±0.3^{b}

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Table.V. Seasonal composition of Total Fatty Acids and (EPA+DHA) of Total Fatty Acids, PC and PE of *P.radiata* (mean ± SE; $n = 6$).

a,b,c.d – values in rows with different letter indexes differ significantly $P \leq 0.05$.

	FA (mg/100g)	TFA	EPA + DHA
Winter	TFA	400.21±50.12[a]	89.75±13.75[a]
	PC	69.71±18.24[c]	21.43±6.09[c]
	PE	32.74±8.57[c]	15.29±4.31[c]
Spring	TFA	334.28±76.8[a]	64.97±15.66[a]
	PC	52.45±14.56[c]	9.43±2.75[c]
	PE	5.04±1.49[d]	0.52±0.16[d]
Summer	TFA	187.06±8.97[a]	34.87±3.03[a]
	PC	6.97±1.18[c]	1.26±0.29[c]
	PE	1.68±0.32[c]	0.13±0.03[c]
Automn	TFA	79.61±6.18[a]	14.39±1.28[a]
	PC	27.69±3.52[b]	3.71±0.34[b]
	PE	15.91±3.03[b]	1.81±0.3[b]