First seasonal investigation of the fatty acid composition in three organs of the Tunisian bivalve *Mactra stultorum*

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**SUMMARY:** This study reveals information for the first time about the total lipid (TL) content and fatty acid composition (FA) of *Mactra stultorum* (*M. corallina*). Three edible organs (foot, mantle and adductor muscle) were the subjects of this research in order to determine the most favorable periods for their consumption in relation to seasonal variability. The results showed lower lipid content in the adductor muscles in summer (12.73 ± 2.55 mg/g dry matter); while a higher content was observed in winter (28.97 ± 3.50 mg/g dry matter). However, similar lipid contents were observed in the mantle and foot tissues among the seasons. The fatty acid composition of *Mactra stultorum* adductor muscles, mantle and foot was dominated by saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA). Palmitic acid (16:0) was the major saturated fatty acid (SFA) and reached higher levels during summer in the foot (26%), mantle (21%) and adductor muscles (25%). Among PUFA, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) showed significant variation among seasons with high levels recorded during winter and spring. Monounsaturated fatty acids (MUFA) did not show any remarkable variation among seasons for the three studied tissues. Seasonal changes in fatty acids were observed for all samples, reaching a maximum level in winter or spring.

**KEYWORDS:** Fatty acid composition; *Mactra corallina*; Season; Total lipid
1. INTRODUCTION

The rayed trough-shell *Mactra stultorum* (= *M. corallina*) (Linnaeus 1758) is a commercially important benthic clam that inhabits sandy bottoms at depths between 5 and 30 m. It is a medium-sized marine bivalve with a very thin and delicate shell with concentric growth lines (Guarniero et al., 2010). *M. stultorum* is distributed along the coastlines of the Black Sea, Mediterranean Sea and the Eastern Atlantic Ocean from Norway to Senegal (FAO, 1987). This species occupies an important trophic level in the food chain, and is a source of food for crustaceans, gastropods and macropage fish (Mnari, 2000). *M. corallina* was reported in Northern (Gulf of Tunisia) and Southern (gulf of Gabes) Tunisian Coasts by Enzenros (2001) and Seurat (1934). This clam is commercially exploited and consumed in the Atlantic Sea (e.g Manche coasts) (Direction Départementale des affaires maritimes de la Manche, 2013), while it is virtually unexploited and not yet commercialized in the Mediterranean Sea.

Sea organisms, in particular, bivalves are considered a good source of omega 3 fatty acids such as eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids that are crucial for human nutrition and health (Shanmugam et al., 2007). It has been well demonstrated that these fatty acids are beneficial for human health and for the prevention of many diseases (coronary artery disease, inflammatory and autoimmune diseases etc.) (Harris and Schacky, 2004; Calder and Grimble, 2002; Gil, 2002). Shellfish are also rich in protein, amino acids, vitamins (e.g. B12), essential elements (Calcium, Magnesium, Zinc...) and are frequently considered a healthy food for human consumption (Ghribi et al., 2018; Dong, 2001).

Lipids are considered essential compounds for the formation of cells, tissue membrane and egg production and are also used in chronic stress (Napolitano et al., 1992; Nevejan et al., 2003). Lipids are a major source of metabolic energy and support animal growth when the food supply from the environment is limited (Holland, 1978). According to Teshima et al., (1988), the distribution of lipids in different organs and tissues depends on their metabolism (digestion, absorption, modification, transport and storage). Lipids and fatty acids in the soft tissue of marine bivalves undergo seasonal fluctuations, which are controlled by exogenous factors (food availability, temperature, salinity etc.) and by endogenous factors (physiological status) (Prato et al., 2010).

Previous studies on *M. stultorum* investigated the Mediterranean Sea (1) and populations (2). They included research on genetics and ecology (Chetoui et al, 2012; et al., 2016) and biology (Chetoui et al., unpublished data).

The lipid content of this species was described by Hanus et al., (2009) in the plasmogen fraction of *Mactra corallina* but no data are available on its fatty acid composition and nutritional value during an annual cycle. To the best of our knowledge, the fatty acid composition and nutritional quality indices in different edible organs (mantle, foot and adductor muscle) of *Mactra stultorum* are examined for the first time.

The aim of this work was to study the seasonal variations in the fatty acid profiles of the *M. stultorum* population from the Tunisian Coasts and to examine the potential influence of environmental parameters and reproductive cycle on its nutritional quality. The results provided by the current study will be useful for indicating periods of the year that are more suitable for the consumption and marketing of *M. corallina* as a new marine resource in Tunisia.

2. MATERIALS AND METHODS

2.1. Animals

Specimens of *Mactra stultorum* (> 3.5cm) were collected over a period of 12 months (from June 2008 to May 2009) from the sandy beach of Kalaat El Andalous (Gulf of Tunisia). The sampling site was located at 37°N 10°67'E (Figure 1). After sampling, *Mactra stultorum* (n=6 each month) were brought to the laboratory in cool boxes. The shells were opened to collect the fresh tissues: adductor muscle (AM), mantle (MT) and foot (FT) and stored at −20 °C until analyses.

At the sampling site, temperature (°C) was measured every month with a thermometer. In the laboratory, chlorophyll *a* was extracted using Whatman GF/F filters with 90% methanol and its concentration was determined according to the method of (Aminot and Chaussepied, 1983).

2.2. Lipid extraction

Lipids were extracted according to Folch et al., (1957) method with a chloroform: methanol (2:1,v/v) solution containing 0.01% butylatedhydroxyl toluene (BHT) as antioxidant. The total lipid content was determined by double weighing and expressed as mg/g DM.

**Fatty acid composition.** The Lipid fraction was transesterified to methyl esters according to Cecchi et al., (1985). Methyl nonadecanoic acid C19:0 (Sigma), which was absent from our samples, was added as internal standard. Methyl esters were analyzed by gas chromatography using a chromatogram “Agilent Technologies” HP 6890 model equipped with a capillary column INNO-WAX (30m x 0.25 μm) and supplied by a carrier gas of nitrogen. The temperature program during the injection was started with a temperature of 50 °C. Thereafter, the T °C was raised to 180 °C (40 °C/min), then to 220 °C (1.33 °C/min). Finally, it was held at 220 °C for 5min.
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.

Identification of FAMEs was based on the comparison of their retention times with those of a mixture of methyl esters PUFA-3 (by SUPELCO) and Marine oil (Mehaden oil by SUPELCO).

Fatty acid peaks were integrated and analyzed using HP chemstation software.

2.3. Data analysis

Data analyses were made using the software Statistica version 5.0. Lipids contents were expressed as mg/g Dry Matter. Fatty acids were expressed in percentages. Data was presented as the seasonal average ± the standard deviation (SD). The averages were compared by the analysis of variance (ANOVA). The Duncan’s test and Pearson correlation were applied. The differences were considered significant when $P < 0.05$. The mean variance in the data set was detected using the principal component analysis (PCA).

3. RESULTS

3.1. Total lipid content in the foot, mantle and adductor muscle of *M. stultorum*

Figure 2 shows the seasonal fluctuations of temperature and chlorophyll *a* content. Temperature values varied between 10.3 °C (winter) and 26.3 °C (summer). The quantity of chlorophyll *a* observed in our study ranged from 2.84 (autumn) to 4.44 mg/l (summer).

Seasonal variations total lipid content (TL) in the three organs of *M. stultorum* are represented in Figure 3. The mantle has the highest amount of TL during all the sampled seasons (> 30 mg/g DM). In the foots, it ranged between 15 and 20 mg/g DM. However, the TL content in the adductor muscle varied significantly among seasons, when the lowest contents were registered in summer (12.73 ± 2.55 mg/g DM) and the highest value was observed during the winter (28.97 ± 3.50 mg/g DM).

3.2. Fatty acid compositions in the foot, mantle and adductor muscle of *M. stultorum*

The seasonal variation in the fatty acid composition of *M. stultorum* is summarized in Table 1. In this study, thirty fatty acids (FA) were identified and three
families were determined. Palmitic acid (C16:0) was the major saturated fatty acid (SFA), ranging between a minimum 17% (in the mantle during winter) and a maximum 26.75% (in the foot during summer). No significant differences were found for C16:0 among organs. However, three FA such as 16:1; 18:1 and C20:1 were considered remarkable MUFA for MT, FT and AM in all the seasons. Nonetheless, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) predominated the polyunsaturated fatty acids (PUFA). For all organs, the values of EPA and DHA ranged from 4 to 10% and from 7% to 20%, respectively.

Non-methylene-interrupted dienoic acids (NMID; 22:2i+22:2j) are considered fatty acid characteristics of bivalve, and were also determined in M. corallina. In addition, NMID were low in the foot (from 1% to 2.60%) compared to the mantle (> 2.60%) and adductor muscle (> 2.30%).

A statistical analysis between the fatty acid composition of M. stultorum and environmental parameters (temperature and chlorophyll a) was tested by ANOVA. The results showed a negative and significant correlation between PUFA and n-3 PUFA and adductor muscles and chlorophyll a.

The variation in fatty acid families among the organs such as SFA, MUFA and PUFA are represented in Figure 4. For all seasons, the M. stultorum foot has the highest values of SFA (comprised between 37% and 49%). While, in the mantle and adductor muscles, SFA were below 40%. Only in winter, did we observe a significant variation (p < 0.05) among organs (Figure a). MUFA percentages in the adductor muscle were constant and comprised between 19 and 20%. Nevertheless, in the mantle and foot, they were higher during the
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### Table 1. (Continued)

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Summer (08)</th>
<th>Autumn (08)</th>
<th>Winter (09)</th>
<th>Spring (09)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20:1</td>
<td>4.07±0.75</td>
<td>6.32±1.68</td>
<td>3.29±0.94</td>
<td>5.54±1.27</td>
</tr>
<tr>
<td>22:1</td>
<td>3.15±0.12</td>
<td>0.86±0.59</td>
<td>1.79±0.32</td>
<td>1.42±0.20</td>
</tr>
<tr>
<td>Σ MUFA 18.34±2.39</td>
<td>20.68±0.43</td>
<td>14.68±0.25</td>
<td>17.52±2.5</td>
<td>16.20±0.29</td>
</tr>
<tr>
<td>Σ SFA 15.35±1.17</td>
<td>17.66±0.23</td>
<td>20.19±0.1</td>
<td>19.92±0.57</td>
<td>19.32±0.86</td>
</tr>
<tr>
<td>Σ PUFA 28.35±2.81</td>
<td>25.90±1.36</td>
<td>20.73±0.48</td>
<td>16.20±0.29</td>
<td>16.1±0.19</td>
</tr>
</tbody>
</table>

Results are given as mean ± SD. *n*=18 each season. Different letters indicate significant differences among seasons for each organ (Duncan’s *P*<0.05). Absence of letters means no significant effect (*P*<0.05).


summer (22% and 26% respectively) and lower in the winter (17% and 14% respectively) (Figure b4). PUFA values for \textit{M. stultorum} were similar in summer and autumn (fig. c4). They increased to reach a maximum in spring in the order of 48.76 ± 9.50%; 43.12 ± 10.26% and 45.05 ± 9.42% for AM, MT and FT respectively (p < 0.05).

Some ratios or lipid quality index were calculated for \textit{M. stultorum} which reflect the nutritional

- Table 2. Lipid quality index of \textit{Mactra stultorum} (foot, mantle and adductor muscle).

<table>
<thead>
<tr>
<th></th>
<th>Foot</th>
<th>Mantle</th>
<th>Adductor muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer (08)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autumn (08)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter (09)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring (09)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFA%</td>
<td>66.8</td>
<td>67.7</td>
<td>67.8</td>
</tr>
<tr>
<td>MUFA%</td>
<td>30.7</td>
<td>32.3</td>
<td>32.2</td>
</tr>
<tr>
<td>PUFA%</td>
<td>2.5</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>EPA+DHA%</td>
<td>14.3</td>
<td>11.3</td>
<td>13.8</td>
</tr>
<tr>
<td>DHA/EPA</td>
<td>1.78</td>
<td>1.87</td>
<td>1.53</td>
</tr>
<tr>
<td>n-3/n-6</td>
<td>2.84</td>
<td>2.16</td>
<td>2.44</td>
</tr>
<tr>
<td>PUFA/SFA</td>
<td>0.61</td>
<td>0.61</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Results are given as mean ± SD, n=18 each season. Different letters indicate significant differences among seasons for each organ (Duncan’s tests, P < 0.05). Absence of letters means no significant effect (P > 0.05).
quality of the flesh (Table 2). Two first ratios were calculated in our study such as EPA+ DHA and EPA/DHA ratios, which were higher during spring with values estimated at 25 and 2%, respectively in all the organs of M. corallina. The PUFA/SFA ratio varied from 0.5 to 1 for all the organs during the 4 seasons; except for the mantle in winter, which increased to 3. Nevertheless, the (n-3) / (n-6) ratio was comprised between 2 to 5 in the three organs.

3.3. Principal component analysis (PCA)

The principal component analysis was performed with respect to the effect of seasonality on the fatty acid composition in the three organs of Mactra. The loading plot is represented in Figure 5A. Factor 1 displayed 16.12% variance, which was defined by SFA (C16:0; C17:0 and C18:0), whereas Factor 2 demonstrated a variance of 13.86% and characterized by C16:1, C20:1, C20:4n-6 and C22:5n-3 variables. The percentages of SFA in the foot, mantle and adductor muscle showed a positive correlation with those of MUFA, which varied similarly during the seasons (except in spring). In contrast, the n-3 PUFA percentages were projected on the negative side of Factor 1 and Factor 2. Figure 5B showed that there is an overlap between two groups: mantle and adductor muscles. This observation confirms the similarity of fatty acid compositions during the 4 seasons. However, the fatty acid composition in the foot formed a separate group.

4. DISCUSSION

The present study showed that the total lipid content observed in M. stultorum was similar to those found in M. verneriformis (Qiaozhen and Qi, 2013) and in M. chinensis (Li et al., 2011) from the Eastern Coast of China.

For aquatic organisms such as bivalves, lipids are considered an important dietary constituent. In stressful environmental conditions and after the depletion of glycogen, the total lipid content is used as an energy source during the reproduction process (Couturier and Newkirk, 1991). In the current work, a significant decrease in TL, especially in the adductor muscles of M. stultorum was observed. We suppose that this reduction could be related to the reproductive cycle of this species.

It is likely that this animal mobilizes the lipids from the muscle to be stored in the gonads for use during the maturation stage. This was confirmed by the reduction in the TL content in M. stultorum during the spawning phase that occurred from summer to autumn (Chetoui, 2016). In contrast, no significant differences were observed in the TL content of the M. stultorum foot and mantle. In general, the digestive gland, gonads and adductor muscles of bivalve species were considered as organs of energy storage during the reproductive cycle (Darriba et al., 2005).

Fatty acids are vital components needed by the body for different metabolic and structural functioning. In our study, the fatty acid compositions in M. stultorum are in agreement with several investigations carried out on M. chinensis (Teshima et al., 1988) and Ruditapes decussates (Pazos et al., 2003). In addition, our results on the dominance of SFA and PUFA in M. stultorum were in agreement with the findings of Teshima et al. (1988) in M. chinensis and Besnard (1988) in P. maximus. Other investigations have demonstrated that n-3 PUFA proportion was accumulated at high levels in the muscle of Megangulus zyonoensis and Megangulus zyonoensis (Kawashima and Ohnishi, 2003). However, SFA was shown to be the more dominant in Glycymeris glycymeris tissues than PUFA and MUFA (Galap et al., 1999).

The seasonal variation in SFA (16:0 and 18:0) in M. stultorum was marked by a significant reduction during spring. This decrease was followed by a slight
increase in MUFA content in all the studied organs. It could be due to the action of delta-9 desaturase that probably transformed SFA to MUFA (e.g. palmitoleic acid (16:1n-7) and Oleic acid (18:1n-9)) (Sprecher, 2000).

Indeed, many studies have shown that the PUFA level in bivalves reflect their good nutritional quality (Fernández et al., 1996).

Other investigations have reported that this important proportion of PUFA probably resulted from their transfer from other reserve tissues such as the gonads and digestive glands to the mantel (Le Pennec et al., 2001; Darriba et al., 2005).

The seasonal variation in ARA for M. stultorum did not present any differences in all seasons among the organs except in winter for the Mantle. This exception was characterized with an increase in the ARA level associated with a decrease in LA level. This was probably due to the stress effects on the animals following a drop in water temperature during this period (10.33 ± 3 °C) (Pernet et al., 2007). In fact, LA is considered the main precursor of ARA, which can confirm their elevation.

n-3 PUFA, including EPA (C20:5n-3) and DHA (C22:6n-3), are considered essential fatty acids and dietary fats which are beneficial to human health (Dong 2001). During the spring, the foot of M. stultorum appeared as the richest organ in EPA and DHA, coinciding with a maximum level of chlorophyll $a$ (4.28mg/l), AM and MT did not show a significant variation during the studied period. The results of essentials fatty acids in our study from the Mediterranean Coast reminded us of those found in M. chinensis from the Pacific Coast (Teshima et al., 1988). Likewise, other authors have shown that an increase in n-3 PUFA; especially EPA and DHA may reduce cardiovascular risk in consumers of marine products by up to 50% (Angerer and Scalaky, 2000). In addition, these fatty acids were of great interest for cardiovascular and some cancer treatments (Fenton et al., 2000).

The edible portion of M. stultorum was found to be rich in these essentials fatty acids (EPA and DHA). Their important nutritional quality was in accordance with other bivalves such as M. chinensis, M. veneriformis and M. murchisoni which are considered important commercial seafood (Ryou, 1997; Wu et al., 2002; Nottingham and White, 2015).

For the Non-methylene-interrupted dienoic (NMID; C22:2i+C22:2j), the adductor muscle and mantle showed important percentages during all periods (> 2%). Fatty acid Non-methylene-interrupted dienoic (NMID) are polyunsaturated fatty acids, of 22 carbon atoms (C22:2i and C22:2j), synthesized by shellfish, in particular bivalves (Zhukova, 1991). Their structures were established as C20:2A5-11, C20:2A5-13, C22:2A7-13 and C22:2A7-15. It was suggested that these fatty acids in animals are derived almost exclusively from food sources and are biochemically inert (Paradis and Ackman, 1975). For M. corallina, levels of C22:2i were lower than those of C22:2j and their sums varied between 1% and 3.74%. These levels were reversed in M. chinensis, which showed higher values which can reach 5% (Hiroaki, 2007). Our results were comparable to those reported in oysters Crassostrea gigas (Dridi et al., 2007); in mussels Mytilus galloprovincialis (Garrido and Medina, 2002) and in pectenidae (Telahigue et al., 2010).

Concerning the lipid quality index, Kraffe et al., (2008) showed that EPA+DHA in Manila clams were closer to our results (14.9%). However, EPA+DHA reflected more functional and structural modifications of mitochondrial membranes of this bivalve than others such as scallops, oysters, and mussels (Kraffe et al., 2008). So, our values for the PUFA/ SFA ratio were higher than those suggested by the Department of Health and Social Security (1994); which were estimated at 0.45. In addition, the (n-3) / (n-6) ratio calculated in our study was close to the one indicated for M. chinensis (Teshima et al., 1988). Compared to other bivalve from the Mediterranean Sea, the n-3/n-6 ratio was higher than those reported for oyster Crassostrea gigas (2.8) and lower than those recorded for the Pectinidae family (Dridi et al., 2007; Telahigue et al., 2010). According to the UK Department of Health, an ideal proportion of n-6/n-3 is recommended at 4.0 (HMSO, 2001). So, a ratio greater than 4.0 was considered harmful to human wellbeing (Andrade et al., 1995). In the present study, n-3/n-6 ratio is greater than 2 and the n-6/n-3 ratio is lower than 1. Therefore, M. stultorum can be considered as part of a healthy diet.

5. CONCLUSIONS

In the light of these results, Mactra stultorum was shown to be a good source for some important fatty acids such as high levels of n-3 PUFA, EPA and DHA. The seasonal variation in fatty acid composition showed a difference among the three studied organs of Mactra stultorum (foot, mantle and adductor muscle). Moreover, spring was considered a remarkable season which was characterized by higher fatty acid levels due to the food availability in the natural environment. According to our results Mactra stultorum may be considered a good resource for manufacturing animal foodstuff and breeding.

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