

RESEARCH ARTICLE

Seasonal variation of biochemical composition of Noah's ark shells (*Arca noae* L. 1758) in a Tunisian coastal lagoon in relation to its reproductive cycle and environmental conditions

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Abstract – The seasonal changes in biochemical composition of the edible bivalve *Arca noae* harvested from a Mediterranean coastal lagoon (Bizerte lagoon, Tunisia) were investigated from October 2013 to September 2014. Potential food sources and nutritional quality indices (NQI) were determined by analyzing the fatty acid profiles of their tissues during an annual reproductive cycle. Results showed that *A. noae* had moisture (73.8–82%) and protein (24.1–58.6% dry weight) as major components, followed by lipid (10.4–28.8% dry weight) and glycogen (4.05–14.6% dry weight). *A. noae* accumulated lipid and glycogen for gonadal development during both maturation periods (late autumn/late spring–summer) to be used during spawning periods (winter/late summer–early autumn). However, proteins were mainly used to support reproductive allocation and played an important role on the energetic maintenance. Lipid and glycogen were found to be significantly related to temperature, salinity and chlorophyll *a* ($p < 0.05$). An increase of condition index (CI) was observed during spring and early summer when both temperature and food supply increased. The total fatty acid (TFA) profile of Ark shells was dominated by polyunsaturated fatty acids (PUFA) (33.8–49.6%), followed by saturated (SFA) (29.1–43.1%) and monounsaturated fatty acids (MUFA) (20.77–28.3%). *A. noae* soft tissues were also rich in macro-minerals (Na, Ca and Mg). The analysis of fatty acid trophic markers revealed that the diet of *A. noae* during the year of study was based on mixture food mainly on phytoplankton followed by zooplankton and other sources (bacteria, seagrass and macroalgae). Furthermore, the nutritional quality indices have revealed that *A. noae* is an excellent source of nutrients and a healthy food for human consumption. These data are useful to the conservation of natural stocks of *A. noae* and the development of sustainable aquaculture production of this shellfish species in the Bizerte lagoon.

Keywords: *Arca noae* / Bizerte lagoon / biochemical composition / fatty acids / macro-minerals / trophic markers

1 Introduction

The marine bivalve «Noah's Ark shell» (*Arca noae* Linnaeus, 1758 (Bivalvia: Arcidae)) is an edible epifaunal bivalve of hard substratum. Its distribution covers the eastern Atlantic Ocean, the Mediterranean and Black Sea (Poutiers, 1987). It lives attached with solid byssus on all types of hard substrate, including rocks or shells, at a depth ranging from approximately the low tide level to over 100 m (Poppe and Goto, 2000) and occurs as solitary individual or in clumps with other species such as *Modiolus barbatus* (Hrs-Brenko and

Legac, 1996). In the Mediterranean, it becomes sexually mature at an early age, 2 years, when slightly larger than 15 mm and 20 mm in males and females, respectively (Bello and Paparella, 2001; Peharda et al., 2006). It reaches 120 mm in length and lives up to 25 years (Puljas et al., 2015). *A. noae* is known as a slow growing species, requiring 3 to 7 years to reach the commercial length of about 50 mm (Peharda et al., 2006). Reproductive cycle and hermaphroditism in *A. noae* has been examined in the Adriatic Sea by Valli and Parovel (1981); Bello and Paparella (2001); Peharda et al. (2006); Bello et al. (2013) and in the South-Western Mediterranean Sea (Bizerte lagoon) by Ghribi et al. (2017). In the Adriatic Sea, *A. noae* reproduces by a single, more or less prolonged gametogenic cycle, either single- or two-peaked spawning. On the contrary,

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A. noae from the Bizerte lagoon shows a rather different pattern of two discrete maturing/spawning phases differing in terms of both length and intensity. The first one starts with a very rapid ovarian maturation phase in October–November, which is followed by a long, slow rate spawning phase extending from November to March. Afterwards, a new ripening phase occurred from April to June/July, which is followed by a short massive spawning event from July to September (Ghribi *et al.*, 2017).

Over the last century, seafood has been in high demand and has become the best alternative for other food sources (e.g. red and white meat) making these products of great economic importance in several countries (Idayachandiran *et al.*, 2014). Bivalves have been known for their high nutritional value; they provide protein, healthy fatty acids (especially EPA (20:5n–3) and docosahexaenoic acid (DHA) (22:6n–3)), vitamins (e.g. B12), amino acids and essential elements (Calcium, Magnesium, Zinc, etc.) and are considered frequently as a healthy food in several dietary regimes (Dong, 2001). Therefore, the seasonal variation on the biochemical composition of bivalve's species has been widely studied (Soudant *et al.*, 1999; Berthelin *et al.*, 2000; Ojea *et al.*, 2004; Hurtado *et al.*, 2012). It is well known that levels of protein, carbohydrate and lipid in the tissue of marine bivalves undergo seasonal fluctuations that are mainly controlled by available food, environmental parameters (in particularly temperature), and the reproductive status (Gabbott, 1983; Idayachandiran *et al.*, 2014). These substrates are usually stored prior to gametogenesis, producing the energy needed for gamete production (Dridi *et al.*, 2007). Among these energetic substrates, fatty acid analysis is becoming increasingly important for investigating trophic interactions in food webs and to provide a longer-term dietary information (Kharlamenko *et al.*, 2001; Dalsgaard *et al.*, 2003; Prato *et al.*, 2010; Ezgeta *et al.*, 2012; Kelly and Scheibling, 2012).

Since only few investigations have been done on the proximate composition (protein, lipid, carbohydrates and fatty acids) and the potential food sources of the Adriatic Sea *A. noae* population (Mali Ston Bay, Croatia) (Dupčić *et al.*, 2014; Ezgeta *et al.*, 2012), studying other populations of the comestible bivalve *A. noae* from different localities is still required, which provides certainly valuable information to the best understanding of the species. In fact, the information obtained from the study of unexploited stocks gives an overview of the natural biological parameters of a species, which are in turn an essential reference tool for the assessment and management of overexploited stocks from other geographical areas (Peharda *et al.*, 2006; Bello *et al.*, 2013).

Compared to the open sea, the Mediterranean Coastal lagoons such as the Bizerte lagoon are known for their peculiar ecosystem, biological diversity, ecological specificity and hydrological characteristics (Cataudella *et al.*, 2015). These differences between the two contrasting environments (Open Sea vs. Lagoon) are mainly related to the relevant differences in thermal regime and trophic status (Dridi *et al.*, 2014). The sea temperature in the Adriatic Sea (in particularly, in Mali Ston Bay) is lower (T: 7.2–25.8 °C; Dupčić *et al.* (2014) than in the Bizerte lagoon (T: 12.9–30.5 °C; Bejaoui *et al.*, 2017). However, the trophic status of the Bizerte lagoon is characterized by a higher primary production of phytoplankton and abundance of food (Grami

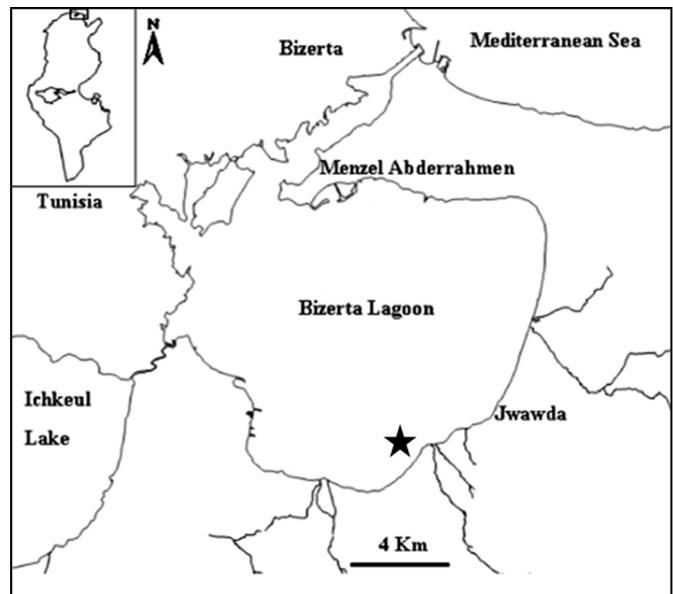


Fig. 1. Map of Bizerte lagoon (Northern Tunisia). The black full star in the Southern part of the lagoon shows the sampling site location.

et al., 2015; Alves *et al.*, 2015), compared to the Adriatic Sea (in particularly, central and southern part) which is characterized by lower phytoplankton biomass and primary production (Fonda-Umani *et al.*, 1990).

The main objective of our study was to provide a detailed analysis of the biochemical composition (lipids, proteins, glycogen, fatty acids and minerals) of *A. noae* during an annual cycle in relation to environmental parameters of a Mediterranean coastal lagoon. All together, they provide information about energetic status, trophic sources and the nutritional value of *A. noae* for human consumption.

2 Material and methods

2.1 Study area

Bizerte lagoon is located on the north of Tunisia, between 37°8′–37°14′N and 9°46′–9°56′E. This coastal lagoon covers an area of 150 km² and the maximal depth is 12 m (Souissi, 1981). This Mediterranean transitional ecosystem is connected directly with the Mediterranean Sea through an 8 km long straight channel and to Ichkeul Lake through a 5 km long (Fig. 1). Five principal streams known as “wadis”, which are Tinja Channel, Rharek, Ben Hassine, Haima and Guenniche are feeding the lagoon non-permanently with freshwaters depending mainly on rainfall. The shellfish aquaculture activities in the lagoon of Bizerte began around the 1950s with Portuguese oyster production (*Crassostrea angulata*). The Tunisian National Office of Fisheries tried in 1958 to grow flat oysters (*Ostrea edulis*) and then in 1972 Pacific oysters (*C. gigas*). Mussel cultures (*Mytilus galloprovincialis*), an autochthonous species of the Bizerte Canal, began in 1963 (Barhoumi, 2014). The sampling station for *A. noae* is located in the southern part (coordinates: 37°08′36.0″N 9°52′20.4″E) of the lagoon far away from urban and industrial sources of pollution but remained influenced by agricultural inputs (Barhoumi, 2014) (Fig. 1).

2.2 Sample collection and processing

A total of 360 sexually mature specimens of commercial size were hand-collected by Scuba diving at about 3 m depth from October 2013 to September 2014, about the 15th day of each month and carried to the laboratory in a cool box. In the laboratory, individuals were placed in running filtered sea water (24 hours at least) for gut-content clearance before being dissected and stored at -20°C until the analyses were carried out. Shells were scraped to remove all epibiotic material. The total weights of all specimens were determined using 0.001 g electronic precision balance. The shell length (SL), height (SH) and width (SW) were measured with a digital calliper to the nearest 0.1 mm below. Ceramic scalpels were used to cut open the arks and remove the soft tissues (byssus excluded) from the shells. For biochemical analysis, the whole body of 10 specimens (53.3–57.6 mm) was dissected per month; each specimen's soft tissue was homogenized using an UltraTurrax[®] blender and then stored at -20°C until analysis. To determine minerals, the whole body of 10 specimens (56.8–58.9 mm) were dissected per month and stored at -20°C until analysis were achieved. 10 specimens (52.4–56.7 mm) were used to determine the condition index (CI) for each monthly sampling.

2.3 Environmental parameters

Data on the basic environmental parameters of Bizerte lagoon surface water such as temperature ($T^{\circ}\text{C}$) and salinity (S psu) were recorded monthly during each sampling period (between 8 am and 9 am) and at the same time the Ark shell collection was done, using a WTW-197i multimeter. Water samples (2 L) were taken from the sampling station every month for the determination of chlorophyll *a* ($\text{Chl } a \text{ mg L}^{-1}$). Chlorophyll *a* was extracted in the laboratory according to standard methods recommended for marine waters (Aminot and Chaussepied, 1983) using Whatman GF/F filters with 90% methanol and concentrations were determined spectrophotometrically at 665 and 750 nm.

2.4 Condition index (CI) and energy value (EV)

The condition index was determined through equation (1) according to Walne (1976):

$$\text{CI} = (\text{Meat dry weight} / \text{dry weight of shell}) \times 100. \quad (1)$$

The energy value (Kcal/g DW), also known as the caloric content, was determined on the base of the dry weight of the biochemical substrates by means of Rubner's coefficients, e.g., lipids 9.45, carbohydrates 4.20, and proteins 5.65 (Winberg, 1971).

2.5 Determination of chemical composition

Moisture content (%) was determined by weight difference after heating the sample at $105 \pm 2^{\circ}\text{C}$ for 24 h (AOAC, 2005). Total lipids were extracted according to the Folch *et al.* (1957) method with the solvent mixture chloroform-methanol (2:1, v/v) containing 0.01% butylated hydroxyl toluene (BHT) as an antioxidant and then were weighted to determine the amount of

lipids. Glycogen was measured by the method of Dubois *et al.* (1956). Protein was determined according to the method described by Lowry *et al.* (1951). The calculation of total protein, lipid, and glycogen was based on the dry tissue weight of each individual and expressed as g/100 g DW (%).

2.6 Fatty acid analyses

Fatty acids from total lipids were transmethylated using the method of Cecchi *et al.* (1985). Nonadecanoic acid (C19:0) (Sigma) (not found in our samples) was added as an internal standard. The resulting fatty acid methyl esters (FAME) were extracted with hexane:diethyl ether (1:1, v/v). The FAMES were subsequently analysed by high resolution gas chromatography (HP, 6890 GC) using a split/splitless injector equipped with a flame ionization detector, and a 30 m HP Innowax capillary column with an internal diameter of 250 μm and a 0.25 μm film thickness. The injector and detector temperatures were maintained at 250°C and 275°C , respectively. The oven temperature was programmed to rise from 50 to 180°C at a rate of $4^{\circ}\text{C}/\text{min}$, from 180°C to 220°C at $1.33^{\circ}\text{C}/\text{min}$ and to stabilize at 220°C for 7 min. Nitrogen was the carrier gas. Identification of FAMES was based on the comparison of their retention times with those of commercial standards methyl esters (SUPELCO polyunsaturated fatty acids (PUFA-3)). Fatty acid peaks were integrated using a software package (HP chemstation software). The relative amount of each FA was expressed as a percentage of the total amount of FA in the analyzed sample.

2.7 Nutritional quality indices

In our study, several nutritional quality indices expressed as ratios and sum were calculated from total fatty acids (TFA) in order to evaluate the nutritional quality of *A. noae*: $n - 3/n - 6$ PUFA ratio was calculated according to Marques *et al.* (2010); EPA + DHA giving by Unusan (2007); PUFA/saturated fatty acid (SFA) and DHA/EPA according to Marques *et al.* (2010) were also determined. Atherogenicity (AI) and Thrombogenicity (TI) indices were evaluated as described by Ulbricht and Southgate (1991). Those two indices aimed to relate the fatty acid profile with the risk of cardiovascular disorders and were defined through equations (2) and (3):

$$\text{AI} = ((4 * \text{C14:0}) + \text{C16:0} + \text{C18:0}) / (\sum \text{MUFA} + \sum \text{PUFA}_{n-6} + \sum \text{PUFA}_{n-3}), \quad (2)$$

$$\text{TI} = (\text{C14:0} + \text{C16:0} + \text{C18:0}) / (0.50 * \sum \text{MUFA} + (0.5 * \text{PUFA}_{n-6}) + (3 * \text{PUFA}_{n-3}) + (\text{PUFA}_{n-3} / \text{PUFA}_{n-6})), \quad (3)$$

where C14:0 is the percentage of myristic acid, C16:0 is the percentage of palmitic acid and C18:0 is the percentage of stearic acid as related to TFA.

2.8 Determination of mineral composition

Macro-minerals such as Ca, Na, and Mg were determined by inductively coupled plasma mass spectrometry (ICP-MS)

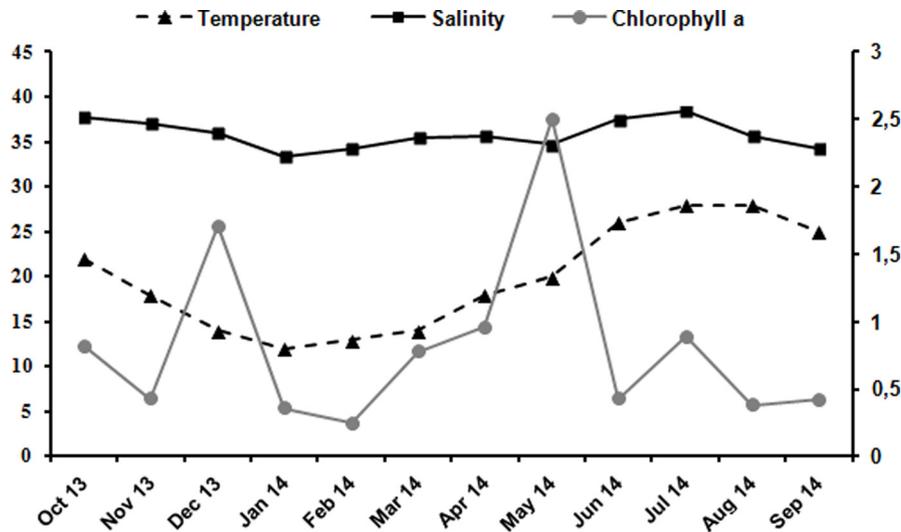


Fig. 2. Physico-chemical parameters of Bizerte lagoon during the sampling period (October 2013–September 2014).

using dynamic reaction cell (DRC) technology (ICP-MS ELAN DRCII, PerkinElmer Inc.). The entire soft tissue of each individual was oven-dried at 105 °C for 24 hours (Carvalho et al., 2000). Then, the dried specimens were ground to powder using an agate mortar. Dried powders were mineralized in Teflon bombs in a closed microwave digestion labstation (Ethos D, Milestone Inc.). The digestion procedure was performed using nitric acid-hydrogen peroxide (HNO₃/H₂O₂ suprapure grade, Merck). Digestates were diluted to an appropriate volume of 50 mL prior to being analyzed. Data were analyzed on a dry weight basis and were expressed in mg/kg⁻¹ DW.

2.9 Fatty acid trophic markers (FATM)

During this study, FATM were used to infer the diet of *A. noae* during an annual cycle. From the fatty acid profile of *A. noae*, various food sources were identified such as: Diatoms: C16:1n-7 (Ackman et al., 1968; Mayzaud et al., 1989; Ezgeta et al., 2012), C20:5n-3 (Mayzaud et al., 1989; Budge and Parrish, 1998); Dinoflagellates: C22:6n-3 /C20:5n-3 (>1) (Dalsgaard et al., 2003), C22:6n-3 (Kelly and Scheibling, 2012); Detrital/Bacterial input: C15:0 + C17:0 + C18:1n-7 (Najdek et al., 2002; Ezgeta et al., 2012), C16:1n-7 (Kelly and Scheibling, 2012); Zooplankton: C18:2n-6 (Mayzaud et al., 1989); C22:6n-3 (Mayzaud et al., 1989; Budge and Parrish, 1998; Kharlamenko et al., 2001); Seagrass, macroalgae and vascular plants: C18:2n-6 (Kelly and Scheibling, 2012).

2.10 Statistical analyses

Data were analyzed using the software STATISTICA 8 (Stat-Soft Inc.) and GraphPad Prism 5 (GraphPad Software Inc.). Results were expressed as means ± standard deviations (SD) and each value was a mean of 10 replications for moisture, protein, glycogen, minerals, condition index and 6 samples for lipid content. After testing the homogeneity and normality of variables using the Shapiro-Wilcoxon test, significant differences between variables were tested with one way analysis of variance (ANOVA) followed by a post-hoc Tukey's test

($p < 0.05$). If conditions for ANOVA were not fulfilled, non-parametric Kruskal–Wallis's test was used ($p < 0.05$). The differences between samples were deemed to be significant at $p < 0.05$. Principal components analysis (PCA) was used to display the relationship between biochemical and environmental parameters among sampling periods.

3 Results

3.1 Environmental parameters

Monthly fluctuations in water temperature (T °C), salinity (S) and chlorophyll *a* concentrations (Chl *a*) at the sampling area are illustrated in Figure 2. The highest water temperature was recorded in summer (28 °C in July and August) and the lowest in winter (12 °C in December). Salinity ranged from 33.4 psu to 38.4 psu, with a minimum in January and a maximum in August (Fig. 2). The monthly variation of Chl *a* concentrations was marked by two important picks, the first one occurred in winter (1.71 mg/L⁻¹) and the second was more pronounced in spring (2.5 mg/L⁻¹) (Fig. 2). The chlorophyll *a* concentrations were related significantly to both T ($p < 0.05$) and S ($p < 0.05$) (Fig. 7). Furthermore, a significant positive correlation between water T and S ($p < 0.05$) was also recorded.

3.2 Biometrics

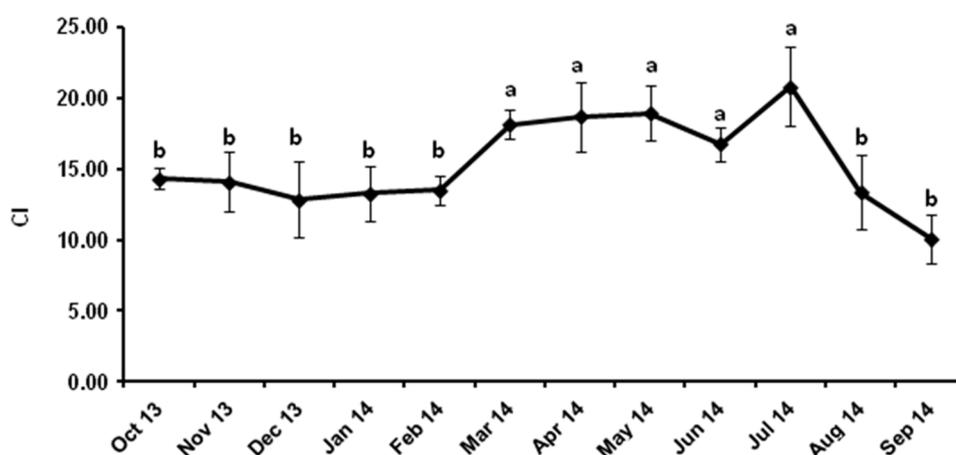
The biometric characteristics (shell length, height and width) are presented in Table 1. The shell length (SL) ranged from 52.35 to 58.20 mm; the shell height (SH) ranged from 27.08 to 31.62 mm and the shell width (SW) varied from 23.77 to 29.54 mm.

3.3 Condition index

Significant differences were observed in the condition index of *A. noae* throughout the year (Kruskal-Wallis test, $H = 82.42$, $DF = 11$, $p < 0.0001$). The maximum value was recorded in July (20.85 ± 2.80) and minimum in September

Table 1. Seasonal biometrical characteristics of *A. noae* shells harvested from Bizerte lagoon during the year 2013–2014 (*n*: number of individuals; SL: shell length; SH: shell height; SW: shell width).

Months	SL (mm)	SH (mm)	SW (mm)
October 2013 (<i>n</i> = 30)	52.35 ± 4.73	27.37 ± 2.53	24.16 ± 2.46
November 2013 (<i>n</i> = 30)	56.54 ± 2.20	30.64 ± 3.65	28.48 ± 3.59
December 2013 (<i>n</i> = 30)	56.51 ± 3.93	31.03 ± 2.70	27.53 ± 2.68
January 2014 (<i>n</i> = 30)	55.77 ± 2.15	31.62 ± 1.52	28.05 ± 2.30
February 2014 (<i>n</i> = 30)	55.68 ± 2.46	30.85 ± 1.66	27.97 ± 1.53
March 2014 (<i>n</i> = 30)	54.28 ± 3.60	27.08 ± 3.84	23.77 ± 2.82
April 2014 (<i>n</i> = 30)	51.44 ± 2.88	29.83 ± 2.43	26.00 ± 1.50
May 2014 (<i>n</i> = 30)	58.20 ± 2.40	30.61 ± 1.97	29.16 ± 2.28
June 2014 (<i>n</i> = 30)	53.55 ± 4.17	29.69 ± 2.04	28.15 ± 1.98
July 2014 (<i>n</i> = 30)	53.19 ± 2.39	27.44 ± 4.84	28.56 ± 3.06
August 2014 (<i>n</i> = 30)	56.42 ± 2.98	29.76 ± 2.12	29.54 ± 2.33
September 2014 (<i>n</i> = 30)	54.84 ± 3.99	31.44 ± 1.73	26.72 ± 3.41

**Fig. 3.** Seasonal variations of *A. noae* condition index (CI) during the study period. Values are expressed as means ± SD (*n* = 10). Different letters indicate significant differences ($p < 0.05$).

(10.09 ± 1.71) (Fig. 3). A positive significant correlation was found between CI and both Chl *a* and S ($p < 0.05$) (Fig. 7).

3.4 Proximate composition and energy value

The proximate composition showed significant seasonal variations marked by phases of storage and depletion of reserves. The fluctuations of moisture (73.8–82%, Kruskal-Wallis test, $H = 70.3$, $DF = 11$, $p < 0.001$); protein (24.1–58.6% dry weight, Kruskal-Wallis test, $H = 70.9$, $DF = 11$, $p < 0.001$); total lipid (10.4–28.8% dry weight, Kruskal-Wallis test, $H = 40$, $DF = 11$, $p < 0.001$) and glycogen (4.05–14.6% dry weight, Kruskal-Wallis test, $H = 65.8$, $DF = 11$, $p < 0.001$) contents are shown in Figure 4. During late autumn, total lipid and glycogen were accumulated in high quantities, while proteins were the lowest. The winter and early spring period was characterized by a dramatic decrease of glycogen and lipid reserves ($p < 0.05$) parallel to the increase of protein content. In fact, protein content increased significantly from December ($p < 0.05$), while total lipid and glycogen increased markedly between March and July, when CI starts to increase and the most important Chl *a* pick

occurred in May. A significant drop in CI levels was observed in June coinciding with a sudden drop of Chl *a* concentration during the same month. During late summer and early autumn, protein and lipid content were maintained in high levels, while glycogen decreased significantly ($p < 0.05$). No statistical correlation was found between protein and both lipid and glycogen content ($p > 0.05$). Meanwhile, total lipid and glycogen levels in Ark shells were significantly positively correlated between each other's ($p < 0.05$) and with the environmental parameters lipid/T ($p < 0.05$); lipid/S ($p < 0.05$); lipid/Chl *a* ($p < 0.05$); glycogen/S ($p < 0.05$) and glycogen/Chl *a* ($p < 0.05$). No correlation was found between protein and the environmental parameters. As regards the energy value (EV), known also as the caloric content, the highest value was observed in June (6.22 Kcal/g DW) and the lowest one in March (3.70 Kcal/g DW) ($p > 0.05$) (Fig. 5).

3.5 Macro-minerals

The levels of major macro-minerals such as sodium (Na), magnesium (Mg) and calcium (Ca) were recorded monthly

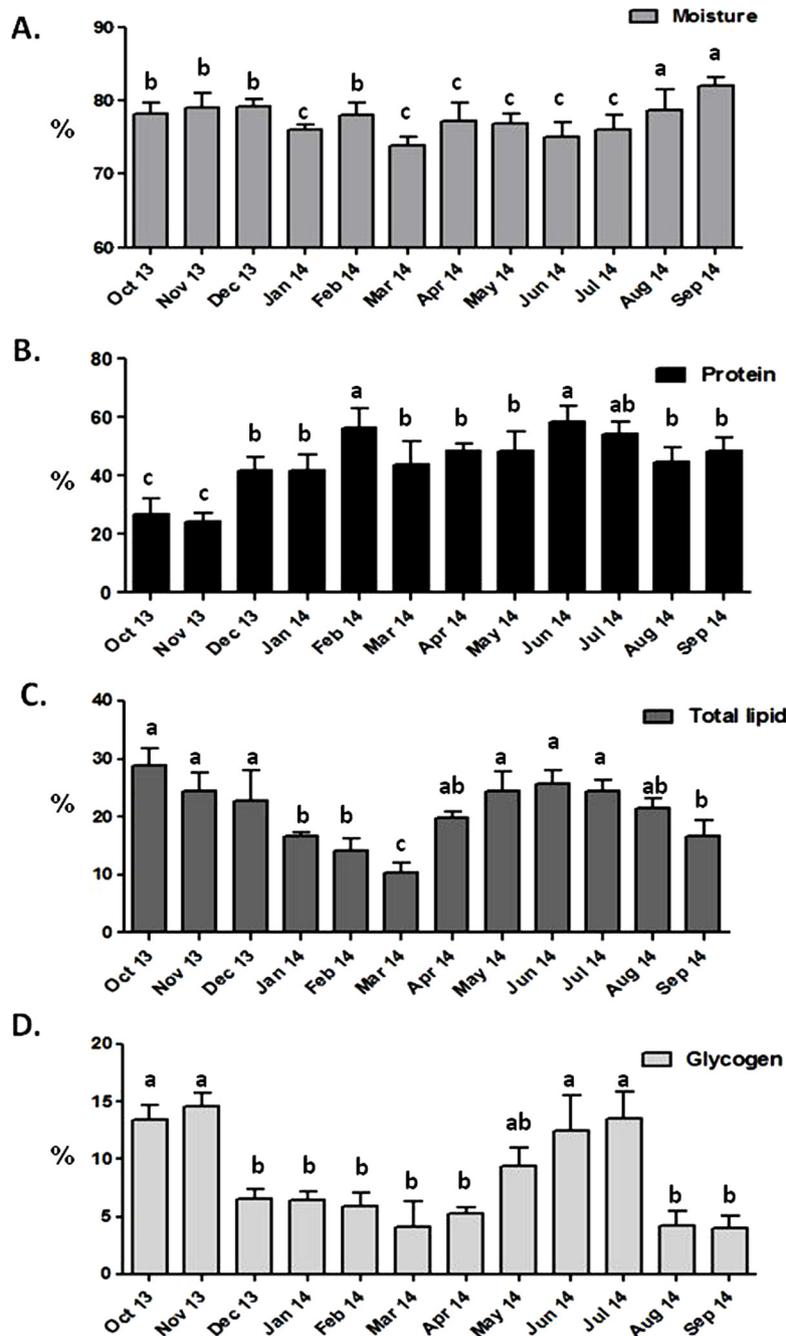


Fig. 4. Proximate composition: A. Moisture percent (%); B. Protein content (%), C. Total Lipid content (%) and D. Glycogen content (%) in *Arca noae* soft tissue from Bizerte lagoon. Different letters indicate significant differences ($p < 0.05$).

(Fig. 6). Na, Mg and Ca whole body levels ranged from 1628 to 36714 mg/kg⁻¹ DW (One-way ANOVA, $F_{(11, 98)} = 16.86$, $p = 0.000$), from 2710 to 5549 mg/kg⁻¹ DW (One-way ANOVA, $F_{(11, 95)} = 23.22$, $p = 0.000$) and from 1530 to 2577 mg/kg⁻¹ DW (One-way ANOVA, $F_{(11, 93)} = 5.54$, $p = 0.000$), respectively (Fig. 6). Na and Mg contents followed a similar pattern and reached the highest levels in July and the lowest in March. The levels of Ca were generally stable through the study period with a slight increase observed from January to September. Significant correlations were observed between macro-minerals, Na was significantly positively correlated to Mg ($p < 0.05$) and to Ca ($p < 0.05$).

Ca and Mg levels were also significantly positively related ($p < 0.05$).

3.6 Fatty acid composition

The seasonal variations in fatty acid composition of the soft tissues are shown in Table 2. Thirty-three fatty acids from myristic (C14:0) to nervonic (C24:1n-9) were detected.

TFA profile of Ark shells was dominated by PUFA followed by SFA and monounsaturated fatty acids (MUFA). Unsaturated fatty acids (UFA) ranged from 56.86% to 72.19%, while SFA ranged from 29.15% to 43.13% (Tab. 2). SFA varied

Table 2. Seasonal variation of the fatty acid composition (% of total fatty acids) of Ark shells collected from Bizerte lagoon.

Fatty acids	Oct-13	Nov-13	Dec-13	Jan-14	Feb-14	Mar-14	Apr-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14
C14:0	4.55 ± 1.28 ^a	5.32 ± 3.38 ^a	4.15 ± 1.46 ^a	4.07 ± 2.15 ^a	5.70 ± 0.63 ^a	6.55 ± 1.32 ^a	5.33 ± 1.53 ^a	5.00 ± 3.79 ^a	8.63 ± 2.06 ^b	7.62 ± 2.19 ^b	6.60 ± 3.62 ^a	5.68 ± 1.33 ^a
C15:0	0.30 ± 0.06 ^b	1.76 ± 2.27 ^b	0.23 ± 0.04 ^a	0.54 ± 0.63 ^a	0.66 ± 0.46 ^a	0.57 ± 0.36 ^a	1.02 ± 0.44 ^b	0.31 ± 0.11 ^a	0.39 ± 0.08 ^a	0.50 ± 0.44 ^a	0.33 ± 0.08 ^a	0.45 ± 0.40 ^a
C16:0	25.46 ± 3.58 ^b	18.81 ± 2.47 ^a	17.45 ± 2.37 ^a	17.79 ± 1.97 ^a	21.32 ± 2.73 ^a	20.55 ± 2.63 ^a	16.03 ± 6.60 ^a	25.34 ± 3.81 ^b	27.64 ± 2.59 ^b	25.28 ± 1.63 ^b	24.99 ± 2.23 ^b	21.06 ± 2.13 ^a
C18:0	6.24 ± 1.30 ^a	6.47 ± 4.37 ^a	4.60 ± 1.10 ^a	5.90 ± 2.61 ^a	5.85 ± 0.77 ^a	5.17 ± 0.58 ^a	4.87 ± 0.71 ^a	5.26 ± 0.31 ^a	5.69 ± 0.98 ^a	5.73 ± 0.60 ^a	5.62 ± 0.89 ^a	6.36 ± 1.07 ^a
C20:0	0.01 ± 0.009 ^a	1.28 ± 0.27 ^b	1.48 ± 0.45 ^b	1.00 ± 0.55 ^b	0.06 ± 0.05 ^a	0.06 ± 0.06 ^a	0.16 ± 0.05 ^a	0.06 ± 0.03 ^a	0.08 ± 0.02 ^a	0.11 ± 0.06 ^a	0.12 ± 0.02 ^a	0.16 ± 0.05 ^a
C22:0	0.22 ± 0.20 ^a	0.29 ± 0.20 ^a	0.35 ± 0.21 ^a	0.35 ± 0.11 ^a	0.59 ± 0.43 ^a	0.82 ± 1.02 ^a	0.99 ± 0.48 ^a	0.30 ± 0.17 ^a	0.34 ± 0.14 ^a	0.94 ± 0.77 ^a	0.60 ± 0.49 ^a	0.63 ± 0.57 ^a
C24:0	0.26 ± 0.44 ^a	0.95 ± 0.37 ^a	0.86 ± 0.32 ^a	1.08 ± 0.61 ^b	0.62 ± 0.84 ^a	0.37 ± 0.32 ^a	1.19 ± 0.84 ^b	0.30 ± 0.26 ^a	0.33 ± 0.18 ^a	0.48 ± 0.38 ^a	0.29 ± 0.10 ^a	0.25 ± 0.19 ^a
SFA	37.07 ± 3.58 ^a	34.93 ± 9.47 ^a	29.15 ± 3.03 ^a	30.76 ± 6.76 ^a	34.84 ± 2.85 ^a	34.12 ± 3.05 ^a	29.62 ± 6.79 ^a	36.60 ± 6.55 ^a	43.13 ± 2.83 ^b	40.70 ± 3.32 ^b	38.58 ± 2.46 ^a	34.62 ± 2.05 ^a
C14:1	0.32 ± 0.18 ^b	1.62 ± 3.65 ^a	0.22 ± 0.20 ^b	0.76 ± 1.14 ^a	0.68 ± 0.51 ^a	0.79 ± 0.97 ^a	0.75 ± 0.26 ^a	1.14 ± 1.98 ^a	0.61 ± 0.45 ^a	0.95 ± 1.07 ^a	1.01 ± 1.08 ^a	0.63 ± 0.60 ^a
C15:1	0.43 ± 0.13 ^a	1.12 ± 0.84	0.84 ± 1.15	0.73 ± 0.15	0.64 ± 0.35	0.70 ± 0.47	0.56 ± 0.20	0.36 ± 0.13	0.57 ± 0.43	0.78 ± 0.77	0.56 ± 0.16	0.50 ± 0.30
C16:1n-7	7.05 ± 1.74 ^b	7.34 ± 2.47 ^b	8.10 ± 3.92 ^b	5.05 ± 0.78 ^a	5.91 ± 2.59 ^a	6.22 ± 1.42 ^{ab}	3.81 ± 1.60 ^a	6.78 ± 0.34 ^a	7.18 ± 1.43 ^b	6.06 ± 1.04 ^{ab}	9.28 ± 2.07 ^b	6.74 ± 1.65 ^{ab}
C18:1n-7	4.87 ± 2.58 ^a	4.87 ± 2.58 ^a	6.63 ± 1.28 ^a	7.58 ± 1.20 ^a	8.17 ± 2.06 ^a	8.21 ± 1.83 ^a	6.35 ± 0.96 ^a	8.88 ± 0.79 ^a	9.67 ± 0.91 ^a	9.41 ± 0.91 ^a	9.85 ± 1.06 ^a	8.09 ± 1.77 ^a
C20:1n-9	3.91 ± 0.69 ^a	5.25 ± 0.92 ^b	6.10 ± 1.39 ^b	5.59 ± 0.95 ^b	4.65 ± 2.58 ^{ab}	2.38 ± 0.93 ^a	2.41 ± 0.43 ^a	2.18 ± 0.28 ^a	2.45 ± 0.65 ^a	3.38 ± 0.84 ^a	3.83 ± 1.25 ^a	5.20 ± 0.85 ^b
C20:1n-7	1.09 ± 0.99 ^a	4.75 ± 0.79 ^b	5.41 ± 1.40 ^b	5.05 ± 1.17 ^b	1.85 ± 0.50 ^a	1.90 ± 1.04 ^a	5.65 ± 7.65 ^b	1.90 ± 1.50 ^a	0.91 ± 0.34 ^a	1.85 ± 1.00 ^a	2.52 ± 2.96 ^a	1.47 ± 0.63 ^a
C22:1	0.09 ± 0.10 ^a	0.61 ± 0.20 ^a	0.60 ± 0.29 ^a	0.57 ± 0.12 ^a	0.07 ± 0.05 ^a	0.18 ± 0.10 ^a	0.32 ± 0.34 ^a	0.12 ± 0.07 ^a	0.07 ± 0.06 ^a	0.20 ± 0.11 ^a	0.12 ± 0.11 ^a	0.17 ± 0.11 ^a
C24:1n-9	0.18 ± 0.23 ^a	0.36 ± 0.29 ^a	0.39 ± 0.19 ^a	0.22 ± 0.14 ^a	0.52 ± 0.40 ^a	0.35 ± 0.17 ^a	0.88 ± 0.69 ^a	0.19 ± 0.20 ^a	3.58 ± 0.73 ^b	0.17 ± 0.15 ^a	0.32 ± 0.34 ^a	0.18 ± 0.08 ^a
MUFA	24.22 ± 3.19 ^a	25.95 ± 4.15 ^a	28.32 ± 3.43 ^a	25.58 ± 1.54 ^a	22.54 ± 7.42 ^a	20.77 ± 1.50 ^b	20.79 ± 6.77 ^b	21.58 ± 2.08 ^a	21.64 ± 1.17 ^a	22.84 ± 1.64 ^a	27.53 ± 3.31 ^a	23.01 ± 2.45 ^a
C16:2	2.54 ± 0.38 ^a	1.84 ± 0.71 ^a	2.68 ± 1.25 ^a	2.49 ± 0.73 ^a	1.76 ± 0.23 ^a	1.53 ± 0.68 ^a	1.32 ± 0.29 ^a	1.81 ± 0.22 ^a	1.91 ± 0.24 ^a	1.79 ± 0.18 ^a	1.53 ± 0.18 ^a	1.72 ± 0.16 ^a
C16:3	0.45 ± 0.16 ^a	0.80 ± 0.41 ^a	0.75 ± 0.52 ^a	0.71 ± 0.30 ^a	0.70 ± 0.44 ^a	0.72 ± 0.51 ^a	1.64 ± 0.55 ^b	0.68 ± 0.14 ^a	0.54 ± 0.15 ^a	0.87 ± 0.24 ^a	0.56 ± 0.10 ^a	1.17 ± 0.94 ^{ab}
C16:4	0.30 ± 0.07 ^a	0.36 ± 0.09 ^a	0.45 ± 0.19 ^a	0.34 ± 0.09 ^a	1.29 ± 0.99 ^{ab}	0.49 ± 0.16 ^a	2.21 ± 0.55 ^b	1.15 ± 0.54 ^a	0.65 ± 0.18 ^a	1.20 ± 0.98 ^{ab}	1.00 ± 0.93 ^{ab}	1.14 ± 0.65 ^{ab}
C18:2n-6	3.79 ± 0.52 ^a	2.14 ± 0.30 ^a	2.67 ± 0.47 ^a	2.77 ± 1.31 ^a	3.44 ± 0.66 ^a	3.35 ± 0.49 ^a	2.44 ± 0.45 ^a	3.38 ± 0.36 ^a	3.21 ± 0.34 ^a	2.88 ± 0.42 ^a	2.91 ± 0.24 ^a	2.20 ± 1.73 ^a
C18:3n-6	0.43 ± 0.49 ^a	0.23 ± 0.07 ^a	0.29 ± 0.07 ^a	0.83 ± 1.36 ^a	0.46 ± 0.36 ^a	0.38 ± 0.15 ^a	0.51 ± 0.13 ^a	0.94 ± 0.24 ^a	1.13 ± 0.21 ^{ab}	0.86 ± 0.16 ^a	0.58 ± 0.13 ^a	2.22 ± 3.54 ^b
C18:3n-3	2.70 ± 0.78 ^a	1.92 ± 0.54 ^a	2.61 ± 0.69 ^a	2.58 ± 0.56 ^a	3.32 ± 0.78 ^b	3.32 ± 0.95 ^b	2.43 ± 0.33 ^a	3.77 ± 0.58 ^b	3.58 ± 0.73 ^b	2.59 ± 0.45 ^a	2.43 ± 0.42 ^a	1.60 ± 0.34 ^a
C18:4n-3	0.34 ± 0.15 ^a	6.47 ± 1.16 ^c	7.25 ± 2.63 ^c	6.00 ± 1.75 ^c	1.90 ± 0.90 ^a	1.62 ± 1.13 ^a	2.13 ± 1.08 ^b	0.59 ± 0.59 ^a	0.42 ± 0.52 ^a	1.90 ± 1.20 ^a	0.77 ± 0.69 ^a	1.23 ± 1.13 ^a
C20:2n-6	0.48 ± 0.05 ^a	1.17 ± 0.23 ^a	0.77 ± 0.20 ^a	1.26 ± 0.24 ^a	0.51 ± 0.41 ^a	1.07 ± 0.80 ^a	0.73 ± 0.42 ^a	0.51 ± 0.17 ^a	0.42 ± 0.084 ^a	0.88 ± 0.80 ^a	0.37 ± 0.13 ^a	0.42 ± 0.18 ^a
C20:3n-6	0.12 ± 0.02 ^a	0.18 ± 0.14 ^a	0.10 ± 0.06 ^a	0.14 ± 0.07 ^a	0.09 ± 0.07 ^a	0.06 ± 0.04 ^a	0.07 ± 0.07 ^a	0.05 ± 0.03 ^a	0.04 ± 0.02 ^a	0.04 ± 0.01 ^a	0.07 ± 0.03 ^a	0.11 ± 0.02 ^a
C20:4n-6	3.15 ± 1.16 ^b	2.61 ± 0.54 ^a	3.17 ± 0.91 ^a	2.84 ± 1.35 ^a	3.08 ± 0.71 ^a	2.98 ± 0.54 ^a	2.57 ± 0.41 ^a	1.95 ± 0.41 ^b	1.44 ± 0.50 ^b	1.71 ± 0.60 ^b	2.38 ± 0.50 ^a	4.34 ± 1.10 ^a
C20:3n-3	0.15 ± 0.10 ^a	0.90 ± 0.24 ^b	1.03 ± 0.34 ^b	0.75 ± 0.41 ^a	0.04 ± 0.04 ^a	0.15 ± 0.08 ^a	0.14 ± 0.08 ^a	0.13 ± 0.04 ^a	0.13 ± 0.03 ^a	0.11 ± 0.06 ^a	0.04 ± 0.04 ^a	0.07 ± 0.02 ^a
C20:4n-3	0.27 ± 0.07 ^a	0.37 ± 0.15 ^a	0.30 ± 0.18 ^a	0.37 ± 0.32 ^a	0.25 ± 0.05 ^a	0.25 ± 0.08 ^a	0.29 ± 0.15 ^a	0.24 ± 0.06 ^a	0.20 ± 0.03 ^a	0.19 ± 0.08 ^a	0.36 ± 0.11 ^a	0.25 ± 0.03 ^a
C20:5n-3	6.63 ± 1.40 ^a	6.30 ± 2.36 ^a	6.037 ± 0.82 ^a	5.62 ± 1.56 ^a	6.52 ± 1.26 ^a	6.59 ± 1.64 ^a	5.19 ± 0.55 ^a	7.68 ± 1.68 ^a	5.16 ± 0.77 ^a	4.21 ± 1.39 ^a	6.11 ± 0.53 ^a	5.78 ± 0.76 ^a
C22:2i/2j	3.80 ± 1.11 ^a	2.98 ± 0.80 ^a	3.85 ± 0.95 ^a	3.66 ± 0.71 ^a	4.72 ± 1.44 ^a	4.47 ± 1.47 ^a	4.87 ± 1.01 ^a	3.50 ± 0.53 ^a	2.38 ± 0.73 ^a	3.62 ± 1.46 ^a	2.55 ± 1.79 ^a	5.75 ± 2.27 ^a
C21:5	0.84 ± 0.26 ^a	0.77 ± 0.36 ^a	0.94 ± 0.26 ^a	0.97 ± 0.19 ^a	0.99 ± 0.40 ^a	1.24 ± 0.35 ^{ab}	2.58 ± 1.11 ^b	1.22 ± 0.34 ^a	0.93 ± 0.31 ^a	1.03 ± 0.33 ^a	1.02 ± 0.38 ^a	1.25 ± 0.21 ^a
C22:3n-3	1.05 ± 0.58 ^a	1.54 ± 0.56 ^a	1.34 ± 0.44 ^a	1.85 ± 0.96 ^a	1.79 ± 1.32 ^a	3.16 ± 2.86 ^b	4.76 ± 3.43 ^b	1.77 ± 0.36 ^a	2.22 ± 0.76 ^{ab}	2.26 ± 1.15 ^{ab}	1.60 ± 0.92 ^a	2.20 ± 1.01 ^a
C22:5n-3	1.20 ± 0.78 ^a	1.77 ± 0.73 ^a	1.58 ± 0.58 ^a	1.86 ± 1.01 ^a	1.51 ± 0.83 ^a	2.50 ± 1.19 ^b	3.29 ± 2.24 ^b	1.65 ± 0.39 ^a	1.66 ± 0.62 ^a	1.47 ± 0.61 ^a	1.87 ± 0.50 ^a	1.89 ± 0.46 ^a
C22:6n-3	10.37 ± 2.16 ^b	6.66 ± 2.35 ^a	7.96 ± 1.86 ^a	8.51 ± 1.50 ^a	10.95 ± 3.09 ^b	11.44 ± 1.41 ^b	12.34 ± 3.56 ^b	10.71 ± 2.62 ^b	9.09 ± 1.57 ^a	8.77 ± 2.20 ^a	7.65 ± 1.75 ^a	9.86 ± 1.66 ^a
PUFA	38.70 ± 6.26 ^a	39.11 ± 6.64 ^a	43.87 ± 4.29 ^a	43.65 ± 5.28 ^a	43.41 ± 6.37 ^a	45.10 ± 4.24 ^a	49.60 ± 9.19 ^a	41.81 ± 5.12 ^a	35.21 ± 3.14 ^b	36.45 ± 4.37 ^b	33.88 ± 4.43 ^b	43.29 ± 4.91 ^a
UFA	62.92 ± 3.58 ^a	65.06 ± 9.47 ^a	72.19 ± 3.69 ^a	69.23 ± 6.76 ^a	65.96 ± 2.79 ^a	65.87 ± 3.05 ^a	70.37 ± 6.79 ^a	63.39 ± 6.55 ^a	56.86 ± 2.83 ^b	59.29 ± 3.32 ^b	61.41 ± 2.46 ^{ab}	66.30 ± 3.18 ^a

Results were given as mean ± SD (*n* = 6). Different letters (a, b, ab and c) in the same row indicate significant differences (*p* < 0.05).

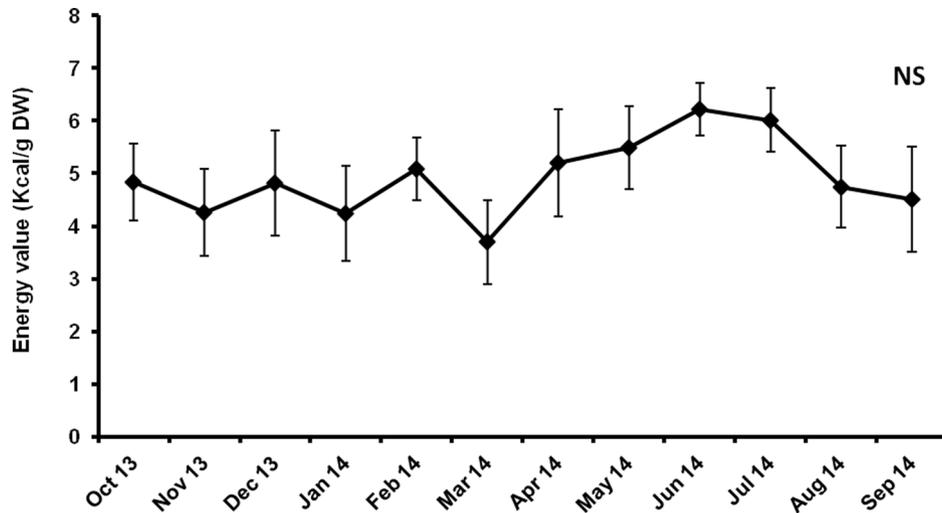


Fig. 5. Seasonal variations of *A. noae* energy value (Kcal/g DW) during the study period. NS indicates non-significant differences ($p > 0.05$).

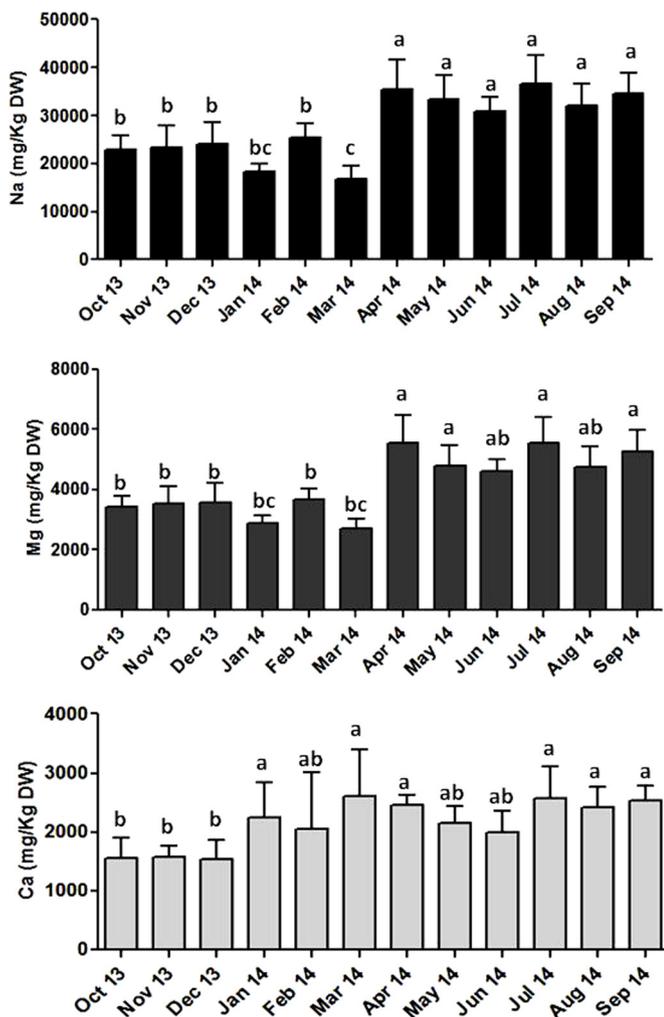


Fig. 6. Seasonal variations of Macro-minerals: A. Na (mg/Kg DW); B. Mg (mg/Kg DW) and C. Ca (mg/Kg DW) in *Arca noae* soft tissue from Bizerte lagoon. Values are expressed as means \pm SD on dry weight basis ($n = 10$). Different letters indicate significant differences ($p < 0.05$).

inversely to PUFA through seasons, during early autumn and summer. SFA increase coincided perfectly with a significant decrease of PUFA ($p < 0.05$) (Tab. 2). MUFA represented the lowest proportion of TFA and were almost stable through the study period except in December and August when a significant increase ($p < 0.05$) in their fraction was observed. The major SFA were palmitic acid (C16:0) followed by myristic (14:0) and stearic (C18:0) acids. The level of MUFA was highly dependent of the levels of oleic (C18:1n-9) and palmitoleic C16:1n-7 acids. However, among PUFA, (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3) prevailed, followed by the non-methylene interrupted dienoic acid (C22:2i/2j) and arachidonic acid (C20:4n-6) (Tab. 2).

3.7 Nutritional quality indices

A comparison between $n-3$ PUFA and $n-6$ PUFA showed that $n-3$ PUFA were in significantly higher proportion (Tab. 3). The highest $n-3/n-6$ ratio was observed during April (4.80) and the lowest during September (2.59). The PUFA/SFA ratio varied between 0.82 (June) and 1.80 (April). DHA/EPA ratio was ranging from 1.06 to 2.38. The sum EPA + DHA was minimal (12.97) in November and maximal (18.39) in May. Low values of AI and TI were observed in December (0.61 and 0.26, respectively) and the highest in June (1.35 and 0.54, respectively) (Tab. 3).

3.8 Fatty acid trophic markers

Table 4 shows the seasonal variation of selected fatty acid (FA) used as trophic markers in order to obtain indications on the potential composition of the diet. Diatoms markers (16:1n-7; C20:5n-3) dominated in autumn, late spring and summer and diminished in winter (January/February). Moreover, a high level of C16:1n-7 ($p < 0.05$) was observed in December coinciding with the first pick of Chl *a*. Dinoflagellates input (C22:6n-3/C20:5n-3 > 1; C22:6n-3) was important throughout the year with significant high levels ($p < 0.05$) noticed in spring season and in the middle of the summer season (C22:6n-3/C20:5n-3 > 1). The tissues

Table 3. Seasonal variation of nutritional quality indices of *Arca noae* from Bizerte lagoon.

	Oct-13	Nov-13	Dec-13	Jan-14	Feb-14	Mar-14	Apr-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14
PUFA/SFA	1.06 ± 0.26 ^a	1.21 ± 0.44 ^a	1.51 ± 0.22 ^a	1.49 ± 0.39 ^a	1.24 ± 0.16 ^a	1.33 ± 0.23 ^a	1.80 ± 0.74 ^a	1.19 ± 9.76 ^a	0.82 ± 0.12 ^b	0.90 ± 0.19 ^b	0.88 ± 0.16 ^b	1.26 ± 0.20 ^a
<i>n</i> -3 PUFA	22.73 ± 4.44 ^{ab}	25.97 ± 4.50 ^a	28.14 ± 3.95 ^a	27.58 ± 4.05 ^a	26.32 ± 4.81 ^a	29.07 ± 4.04 ^a	30.60 ± 8.28 ^a	26.58 ± 4.51 ^a	22.50 ± 2.34 ^{ab}	21.54 ± 1.90 ^b	20.78 ± 3.03 ^b	22.92 ± 3.56 ^{ab}
<i>n</i> -6 PUFA	8.00 ± 0.99 ^a	6.35 ± 0.89 ^a	7.02 ± 1.45 ^a	7.86 ± 1.00 ^a	7.60 ± 0.91 ^a	7.55 ± 0.60 ^a	6.35 ± 0.87 ^a	6.85 ± 0.44 ^a	6.27 ± 0.54 ^a	8.19 ± 1.92 ^a	6.32 ± 0.77 ^a	9.31 ± 2.32 ^a
DHA/EPA	1.57 ± 0.22 ^a	1.06 ± 0.20 ^a	1.31 ± 0.19 ^a	1.55 ± 0.27 ^a	1.72 ± 0.63 ^a	1.79 ± 0.37 ^a	2.38 ± 0.70 ^b	1.39 ± 0.28 ^a	1.77 ± 0.33 ^a	2.16 ± 0.51 ^b	1.24 ± 0.18 ^a	1.72 ± 0.35 ^a
EPA + DHA	17.00 ± 3.44 ^{ab}	12.97 ± 4.58 ^a	14.00 ± 2.58 ^a	14.14 ± 2.86 ^a	17.47 ± 3.57 ^{ab}	18.04 ± 2.64 ^b	17.53 ± 3.71 ^b	18.39 ± 0.41 ^b	14.26 ± 2.03 ^a	12.98 ± 3.34 ^a	13.77 ± 2.27 ^a	15.64 ± 1.88 ^a
<i>n</i> -3/ <i>n</i> -6	2.84 ± 0.42 ^b	4.08 ± 0.34 ^a	4.07 ± 0.54 ^a	3.57 ± 0.87 ^a	3.45 ± 0.51 ^a	3.84 ± 0.46 ^a	4.80 ± 1.01 ^a	3.87 ± 0.52 ^a	3.59 ± 0.31 ^a	2.77 ± 0.80 ^b	3.31 ± 0.42 ^a	2.59 ± 0.71 ^b
AI	0.91 ± 0.17 ^a	0.84 ± 0.43 ^a	0.61 ± 0.16 ^a	0.67 ± 0.31 ^a	0.89 ± 0.13 ^a	0.91 ± 0.18 ^a	0.75 ± 0.29 ^a	0.95 ± 0.39 ^a	1.35 ± 0.23 ^b	1.21 ± 0.18 ^b	1.04 ± 0.25 ^{ab}	0.91 ± 0.12 ^a
TI	0.44 ± 0.10 ^a	0.34 ± 0.15 ^a	0.26 ± 0.05 ^a	0.29 ± 0.13 ^a	0.35 ± 0.03 ^a	0.34 ± 0.06 ^a	0.28 ± 0.12 ^a	0.40 ± 0.13 ^a	0.54 ± 0.07 ^b	0.51 ± 0.07 ^b	0.48 ± 0.09 ^b	0.41 ± 0.05 ^a

Results were given as mean ± SD (*n* = 6). Different letters (a, b and ab) in the same row indicate significant differences (*p* < 0.05).

contained high proportions of zooplankton markers (C18:2*n* - 6; C22:6*n* - 3). However, a shift toward higher level of zooplankton (C22:6*n* - 3) was observed in late winter and spring (*p* < 0.05). Detrital and bacterial FA markers (C15:0 + C18:1*n* - 7; 16:1*n* - 7) predominated late spring and summer seasons, when water T levels increase. Moreover, C15:0 + C18:1*n* - 7 sum showed a significant increase during October (*p* < 0.05) as well as the proportion of 16:1*n* - 7 increased in the same period. Seagrass, macroalgae and vascular plants inputs were also maintained at high proportions during all seasons (C18:2*n* - 6).

3.9 Principal component analysis (PCA)

The first two factorial axes (PC1 (34.22%) and PC2 (20.18%)) explained 54.40% of the total variance (Fig. 7A). The first component (PC1) was characterized by high positive contributions of PUFA, *n* - 3 PUFA and negative contribution of SFA, TI, AI, T and S. However, MUFA boasts the strongest inertia on axis 2 which also represented negative loads of Protein, Ca, DHA, EPA + DHA and CI. The projection of individuals (each samples from each month) on the same factorial plan (1:2) showed that the different cases could be clustered into two groups (I and II) (Fig. 7B). Group I, which was composed of individuals sampled during June and July, showed a negative contribution on the first component (PC1). This correlation coincided with high SFA, AI, TI, T and S and low PUFA and *n* - 3 PUFA. However, group II, represented by individuals sampled during winter and spring was characterized by high PUFA, especially *n* - 3 PUFA, DHA and EPA + DHA (Fig. 7B). In fact, the first principal component (PC1) opposes group I, negatively correlated with this axis, to group II.

4 Discussion

Our study is the first investigation of the nutritional quality of wild Noah's Ark shell *A. noae* from a Mediterranean lagoon in Tunisia. It gives the average seasonal changes in biochemical constituents and in fatty acid composition of the whole animal tissues in relation to the annual reproductive cycle and to the environmental conditions (T, S and Chl *a*).

During this study, CI reflects in part the evolution of the sexual cycle of *A. noae*. Its variation seems to be correlated to the second sexual cycle which occurred from April to September (Ghribi et al., 2017). Similar results were found in some other bivalve species showing that CI did not follow exactly the evolution of their reproductive cycle (Matias et al., 2013; Boussoufa et al., 2015). However, Peharda et al. (2006) found that CI reflected perfectly the reproductive cycle of *A. noae* from Mali Stone Bay (Adriatic Sea, Croatia).

The soft tissues of *A. noae* were characterized by high protein content followed by lipid and glycogen levels. High protein levels were observed through the study period, except during autumn, when protein values decreased. This decrease was offset by the presence of high lipid and glycogen content. In period of reduced glycogen contents (from December to April, August/September) and low Chl *a* levels, proteins were at their highest levels suggesting their important contribution to the energetic maintenance. According to Galap et al. (1997),

Table 4. Fatty acids, fatty acid ratios and sum that were used as trophic markers to identify *A. noae* diet in the Bizerte lagoon.

FATM/Source	References	Oct-13	Nov-13	Dec-13	Jan-14	Feb-14	Mar-14	Apr-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14
C16:1 <i>n</i> -7	Ackman et al. (1968)	7.05	7.34	8.10	5.05	5.91	6.22	3.81	6.78	7.18	6.06	9.28	6.74
Diatoms	Mayzaud et al. (1989)	±1.74 ^b	±2.47 ^b	±3.92 ^b	±0.78 ^a	±2.59 ^a	±1.42 ^{ab}	±1.60 ^a	±0.34 ^b	±1.43 ^b	±1.04 ^{ab}	±2.07 ^b	±1.65 ^{ab}
Bacteria	Ezgeta et al. (2012)	6.63	6.30	6.037	5.62	6.52	6.59	5.19	7.68	5.16	4.21	6.11	5.78
C20:5 <i>n</i> -3	Kelly and Scheibling (2012)	±1.40 ^a	±2.36 ^a	±0.82 ^a	±1.56 ^a	±1.26 ^a	±1.64 ^a	±0.55 ^a	±1.68 ^a	±0.77 ^a	±1.39 ^a	±0.53 ^a	±0.76 ^a
Diatoms	Mayzaud et al. (1989)	1.57	1.06	1.31	1.55	1.72	1.79	2.38	1.39	1.77	2.16	1.24	1.72
C22:6 <i>n</i> -3/ C20:5 <i>n</i> -3 > 1	Budge and Parrish (1998)	±0.2 ^a	±0.2 ^a	±0.2 ^a	±0.3 ^a	±0.6 ^a	±0.4 ^a	±0.7 ^b	±0.3 ^a	±0.3 ^a	±0.5 ^b	±0.2 ^a	±0.3 ^a
Dinoflagellates	Dalsgaard et al. (2003)	10.37	6.66	7.96	8.51	10.95	11.44	12.34	10.71	9.09	8.77	7.65	9.86
C22:6 <i>n</i> -3	Mayzaud et al. (1989)	±2.16 ^b	±2.35 ^a	±1.86 ^a	±1.50 ^a	±3.09 ^b	±1.41 ^b	±3.56 ^b	±2.62 ^b	±1.57 ^a	±2.20 ^a	±1.75 ^a	±1.66 ^a
Zooplankton	Budge and Parrish (1998)	3.79	2.14	2.67	2.77	3.44	3.35	2.44	3.38	3.21	2.88	2.91	2.20
Dinoflagellates	Kharlamenko et al. (2001)	±0.52 ^a	±0.30 ^a	±0.47 ^a	±1.31 ^a	±0.66 ^a	±0.49 ^a	±0.45 ^a	±0.36 ^a	±0.34 ^a	±0.42 ^a	±0.24 ^a	±1.73 ^a
C18:2 <i>n</i> -6	Kelly and Scheibling (2012)	11.4	6.63	6.86	8.12	8.83	8.78	7.37	9.19	10.1	9.9	10.2	8.54
Zooplankton	Najdek et al. (2002)	±7.7 ^b	±2.2 ^a	±4.5 ^a	±5 ^a	±5.3 ^a	±5.4 ^a	±3.8 ^a	±6.1 ^b	±6.6 ^b	±6.3 ^b	±6.7 ^b	±5.4 ^a
Seagrass	Ezgeta et al. (2012)												
Macroalgae													
Vascular plants													
Detritus/Bacterial													
C15:0 + C18:1 <i>n</i> -7													

Different letters (a, b and ab) in the same row indicate significant differences ($p < 0.05$).

exhausted. *A. noae* from the Bizerte lagoon seems to be able to modulate the accumulation and depletion of the energetic substrates during its annual continuous reproductive activity. Furthermore, we have noticed that the second period of gonad maturation in *A. noae* (Ghribi *et al.*, 2017) was initiated with the accumulation of reserve materials when high food supply occurred (Chl *a*), indicating that the newly ingested food energy is mainly used by the animal to fuel the production of gametes. The stored energy reserves during gonad maturation will be, then, rapidly depleted during spawning (Park *et al.*, 2011). The seasonal variation of the mean caloric content of Ark shell tissues reflected those of lipid and glycogen. In fact, high values were reached during late spring and summer, period of gonad development and decreased in August and September (spawning period) (Ghribi *et al.*, 2017). Higher energy contents were observed during the second reproductive cycle. Pazikowska and Szaniawska (1988) have also reported high caloric content in mussels from the Baltic Sea during gonad development. We suggest that during gonadal development of the first cycle (Ghribi *et al.*, 2017), the low energy value observed in *A. noae* was mainly affected by protein decrease. Similar energy values were found in *M. edulis* from Scottish Sea (Okumus and Stirling, 1998) and *D. trunculus* from the Gulf of Tunis (Boussoufa, 2014).

During the present study, the levels of Na, Mg and Ca in *A. noae* from Bizerte lagoon were lower than values reported in *D. incarnatus* (Periyasamy *et al.*, 2014) and *Parreysia cylindrica* (Swapna and Ravinder, 2015). Ca levels were mostly stable through the year, meanwhile Na and Mg varied significantly and reached highest values during spring and summer. This increase can be attributed either to the richness of aquatic environment in these elements during this period or the increase in the filtration rate of Ark shells as a result of the temperature rise (George *et al.*, 2013). In human nutrition, minerals constitute an important component of hormones, enzymes and enzyme activators indicating that they are involved in the building and functioning of organisms (Periyasamy *et al.*, 2014). Overall, the soft tissue of *A. noae* from the Bizerte lagoon can be considered as good source of interesting nutritional minerals for human diet. Generally, the amount of accumulated minerals may vary depending on the mineral composition of the sea water and changes in the species' diet (Chakraborty and Joseph, 2015). Thus, we have observed positive significant correlations between these elements suggesting their similar accumulation behavior (George *et al.*, 2013).

It is well known that endogenous factors (reproduction, etc.) and/or exogenous factors (temperature, food availability, etc.) are responsible for the variation of fatty acids in aquatic organisms (Ojea *et al.*, 2004; Dridi *et al.*, 2007). The fatty acid profile of *A. noae* from the Bizerte lagoon showed predominance of unsaturated fatty acids over saturated fatty acid through the study period. A high degree of unsaturation was characteristic of a healthy marine mollusc (Dupčić *et al.*, 2014). PUFA contents in *A. noae* soft tissue begin to rise from December to May and reached their highest values in April coinciding with the increase of Chl *a* levels. Meanwhile, SFA content was significantly higher during the warmer season (summer). Our results showed that PUFA levels varied inversely to SFA ($p < 0.05$), suggesting that the unsaturation degree decreased during warm season and increased during

cold period. In fact, statistical analysis showed that T was correlated positively to SFA and negatively to PUFA ($p < 0.05$). These findings are in concordance with those found in *M. galloprovincialis* (Irisarri *et al.*, 2014) from Spanish coasts. According to Hall *et al.* (2002), high PUFA content in bivalve's cells during cold period (low water temperature) is needed to maintain membrane fluidity. It is well known that PUFA is a source of energy and function as components of cell membranes, modulators of gene expression and precursors for eicosanoids (Idayachandiran *et al.*, 2014). As temperature is known to alter the membrane fluidity, protein structure, rates of diffusion and velocity of chemical reactions (Hochachka and Somero, 2002), the change in the fatty acid composition of *A. noae* tissues, which was significantly affected by T, could be probably due to an adaptation process known as homeoviscous adaptation (HVA). This adaptation is represented by remodeling membrane lipids by changes in phospholipids, fatty acid and cholesterol content that compensate for the effect of T °C on membrane structure (Hazel, 1995). *A. noae* seems to counteract this temperature effect, like any poikilotherms species, by the decrease in the unsaturated fatty acids when temperature increased. Likewise, Pernet *et al.* (2007) showed that the unsaturation index in the membrane phospholipids of the gills of both species *C. virginica* and *M. edulis* decreased during warming experimental conditions.

The major fatty acids identified in *A. noae* were palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), EPA (C20:5 $n - 3$) and DHA (C22:6 $n - 3$). These findings were in agreement with those reported for *A. noae* in Mali Ston Bay (Dupčić *et al.*, 2014) and for *D. trunculus* in the Gulf of Tunis (Boussoufa *et al.*, 2011). During this study, PUFA, MUFA and SFA levels observed in *A. noae* were in the same range of values reported by Dupčić *et al.* (2014) and Ezgeta *et al.* (2012) in *A. noae* from Mali Ston Bay (Adriatic Sea).

In general, *A. noae* soft tissue was richer in $n - 3$ PUFA than $n - 6$ PUFA. The levels of EPA + DHA sum, $n - 3/n - 6$, PUFA/SFA and DHA/EPA ratios were high all over the year; while, the AI and TI indices, known to reflect the atherogenic and thrombogenic potential of fatty acids (Hosseini *et al.*, 2014), were low during this study. These results showed the good nutritional quality of *A. noae* tissues. Our findings are in agreement with previous studies on bivalve's nutritional quality indices (Orban *et al.*, 2002; Boussoufa *et al.*, 2011; Dupčić *et al.*, 2014). The consumption of marine food, rich in $n - 3$ PUFA, is recommended for its benefits for human health and for prevention against many diseases (coronary artery disease, inflammatory and autoimmune disorder, etc.).

The seasonal variation of the potential food sources of *A. noae* from the Bizerte lagoon showed that phytoplankton is a major one dominated by dinoflagellates and diatoms all over the year, followed by zooplankton and other important sources such as detritus/bacteria input, seagrass, macroalgae and vascular plants. These results are in agreement with the findings of Ezgeta *et al.* (2012) on the food sources of *A. noae* from Adriatic Sea. The mixed diet observed in *A. noae* over the course of study; depend probably on the availability of food in their natural habitat and filtration rate. Previous studies on the structure and functioning of the planktonic food web in the Bizerte lagoon showed the continuous supply and availability of

microphytoplankton (diatoms), microzooplankton, bacteria and detritus (Sakka *et al.*, 2007; Grami *et al.*, 2015) in the lagoon waters. Bivalves can also feed on macroalgae particles, seagrass and vascular plants (Perez *et al.*, 2013; Gaillard *et al.*, 2017). According to Zaabar *et al.* (2015), the vegetal composition of Bizerte lagoon is characterized by the presence of various seagrass and macroalgae species (e.g. *Cymodocea nodosa*, *Cladophora sp.*, *Ulva lactuca*, *Gracilaria bursa-pastoris*) all the year. The relative contribution of different dietary components on the bivalve diet's varied in each species due to their selection of food (based on particle size, nutritive value etc.), their position in the water column, the filtration rate and seasonal changes of food sources in water (Defossez and Hawkins, 1997; Beninger *et al.*, 2007; Ezgeta *et al.*, 2012). Either in maturation or spawning phase, *A. noae* seems to use a mixed food from its environment. During this study, we did not observe a clear preference for a particular food source during a specific period. Although zooplankton markers seem to increase slightly on depends of diatoms and dinoflagellates markers during late winter and early spring. We suppose that the seasonality of these trophic markers is mainly influenced by environmental parameters ($T^{\circ}\text{C}$) and food supply on the natural habitat.

5 Conclusion

As compared to a previously studied *A. noae* population from the Adriatic Sea, *A. noae* from Bizerte lagoon displayed a different cycle of energy storage and utilization, which was closely related to the reproductive cycle of the species and environmental parameters, in particularly, temperature and chlorophyll *a*. *A. noae* harvested from Bizerte lagoon may be considered as an interesting marine resource and healthy seafood product with interesting dietetic properties. Important protein content, high unsaturated fatty acids, good intake of DHA and EPA, macro-minerals, healthy nutritional quality indices indicate the great nutritional value of the species. Furthermore, the analysis of fatty acid trophic markers showed that *A. noae* from Bizerte lagoon has the ability to use simultaneously a mixed food sources available in its environment with a preference for phytoplankton. These preliminary data on the seasonal variation of biochemical composition and nutritional quality of the unexploited Ark shell *A. noae* from Bizerte lagoon provide an important basis for its economic valuation and to promote its consumption as new marine resource in Tunisia.

Disclosure of interest

The authors report no conflicts of interest.

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