RESEARCH ARTICLE

Trace elements and oxidative stress in the Ark shell *Arca noae* from a Mediterranean coastal lagoon (Bizerte lagoon, Tunisia): are there health risks associated with their consumption?

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Abstract

The current study examined the concentrations of ten trace elements (TE) (nickel, chromium, cadmium, iron, zinc, manganese, aluminum, copper, selenium and lead) in the edible tissue of the Ark shell *Arca noae* (L. 1758) from a Mediterranean coastal lagoon, the Bizerte lagoon during 2013–2014. The analysis of several redox status biomarkers, metallothioneins (MTs), malondialdehyde (MDA), glutathione peroxidase (GPx), reduced glutathione (GSH) and acetylcholinesterase (AChE), was monitored as a response to TE bioaccumulation and environmental parameters variability. Significant differences ($p < 0.05$) were observed between mean seasonal TE concentrations in *A. noae* soft tissue. The highest TE concentrations in *A. noae* soft tissues were recorded during summer, which coincided with the increase of body dry weight (BDW) and the gonad index (GI). During this season, biomarker responses were enhanced, revealing significant increases of MTs, MDA and GSH levels as well as GPx activity in *A. noae* tissues, while a decrease of AChE activity was observed. The levels of TE analyzed in *A. noae* and several parameters used to assess the potential human risk (estimated weekly intake, target hazard quotient and target hazard risk) were lower than the permissible limits for safe seafood consumption. Consequently, this shellfish can be considered safe for human consumption. This preliminary study presents prospects for the valorization of this seafood product in Tunisia's food sector. It also gives basal information for future environmental assessment studies in which *A. noae* could be used as early warning tools in the field of biomonitoring programs and confirms the usefulness of biomarkers to monitor the health status of aquatic organisms.

Keywords *Arca noae* · Southern Mediterranean Sea · Trace elements · Contamination · Redox status · Human health

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Introduction

Estuaries and coastal lagoons known as highly productive areas are considered to be of extreme ecological and economic value (Bejaoui et al. 2017). Those ecosystems reinforce vital habitats for aquatic organisms and support many human activities such as fisheries, urban and industrial development and tourism. However, agricultural, urban and industrial discharges resulting from human activities can lead to high levels of pollution in these coastal habitats (Barhoumi et al. 2014; D'Adamo et al. 2008) that can affect living organisms and change their communities and the global ecological functioning of ecosystems (Warwick 2005). Among coastal lagoons, we quote the Bizerte lagoon (northern Tunisia), one of the first local ecosystems for fishing and mussels and other bivalve species production in Tunisia. Shellfish farming activities began in the 1950s with oyster farming (*Crassostrea angulata*). The Tunisian National Fisheries Office tried in 1958 to raise

the oyster *Ostrea edulis* then, in 1972, the oyster *Crassostrea gigas*. *Mytilus galloprovincialis* culture, an indigenous species of the Bizerte lagoon, began in 1963 (Barhoumi 2014). Unfortunately, in recent decades, a sharp decline in its fisheries resources has been observed. The lagoon receives water laden with urban waste from neighboring cities as well as industrial waste. Industrial activities are highly developed near the lagoon and chronically expose aquatic organisms to xenobiotics such as organochlorine compounds (OCs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCBs) and trace elements (TEs) (Trabelsi and Driss 2005).

Trace elements (TE) in coastal systems are a major problem as these environments, are frequently used for the removal and dilution of several wastes and thus contribute to the regular degradation of the environment because of their continuous inputs, persistence, toxicity and ability to concentrate in organisms through food web (Spencer et al. 2006; Wang et al. 2009; Ali et al. 2013). A wide variety of TE can attain coastal waters via numerous discharges ways such as industrial, urban and agricultural (Waeles et al. 2007; Viegas et al. 2009). The effect of toxic and persistent pollutants on the marine ecosystem can be expressed in terms of biochemical endpoints (e.g. biomarkers of toxicity) (Kamel et al. 2014; Mahmoud et al. 2010). Biomarker responses can be influenced by seasonal changes of both abiotic (environmental parameters and food availability) and biotic factors (metabolic activities such as reproductive cycle) (Bocchetti and Regoli 2006).

Bivalves, like any other marine organisms, can be exposed to many chemicals and can bioaccumulate xenobiotics. However, because of their resistance to harvesting stress and exposure to polluted environments, their sedentary nature and their natural abundance (Boussoufa, 2014), bivalves are designed as sentinel species and used in several biomonitoring programs (Bejaoui et al. 2017; Goldberg 1975; Richir et al. 2014).

The Bizerte lagoon inhabits a wide diversity of marine invertebrates, among them the valuable shellfish *Arca noae*. Like any benthic invertebrates, this mollusk may accumulate TE whether essential or not, which causes toxic effects above threshold levels. *A. noae*, an edible bivalve of the family Arcidae, lives attached on rocky grounds by solid byssus, from the low tide level to depth of about 120 m (Hrs-Brenko and Legac 1996). It is widespread in the Mediterranean Sea and the eastern Atlantic Ocean from Portugal to Angola (Gofas 2008). *A. noae* reaches 120 mm in length and lives up to 25 years (Puljas et al. 2015). *Arca noae* represents one of the important shellfish species that are commercially exploited throughout the eastern Adriatic Sea (Zupan et al. 2012) and reaches high market quotations in Croatia, Italy and Slovenia (Poutiers 1987).

Recent studies on *A. noae* from the eastern and central Adriatic coasts (Croatia) have shown that this shellfish may accumulate various trace elements, polychlorinated biphenyls and organochlorine pesticides (Ivankovic et al. 2016; Milun et al. 2016; Erk et al. 2018). Marine bivalves are known to pump large amounts of seawater and therefore come into contact with various chemicals in the water. Many chemical contaminants can cause potential toxicity, including inorganic chemicals (TE such as Cd, As, Hg and Pb) and organic compounds (polychlorinated biphenyls [PCB], dioxins and chlorinated hydrocarbons [PAHs]). These contaminants are accumulated in bivalve tissues both by absorption of the dissolved phase and by ingestion (Wang, 2009). Generally, the accumulation of TE and organics in mollusks is likely affected by environmental and seasonal/chemical factors and physiological processes (ODEQ, 2007).

Two other short reports published by Ozretic´ et al. (1990) and Cuculic´ et al. (2010) explored the possible correlation between some TE concentrations in *A. noae* tissues and the quality of its environment. All these studies concerned only *A. noae* populations from the Adriatic Sea. No data is available concerning the concentrations of TE in Ark shells from the Southern Mediterranean coasts. Similarly, there are little studies on biomarkers of oxidative stress in this species. De Clercq (2016) has reported the seasonal variation in metallothionein (MT) levels of *A. noae* from the Adriatic Sea. While, Peric et al. (2013) have demonstrated the effect of in vitro contamination with an organophosphorus pesticide (trichlorfon) on cholinesterase (ChE, EC 3.1.1.7) activity in the gills and adductor muscle of *A. noae*.

In the current work, the impact of each TE on consumer health is considered through several parameters: estimated weekly intake, target hazard quotient and target hazard risk. Considering that environmental parameters (temperature, salinity, pH, chlorophyll *a* and suspended particulate matter) may influence biological responses and TE bioaccumulation, we follow those parameters together with *A. noae* biotic parameters (reproductive cycle and body dry weight) for a proper interpretation of the data trying to answer the following questions: Does *A. noae* harvested from Bizerte lagoon accumulate trace element (TE) beyond threshold recommended for human consumption? Are they influenced by the physicochemical parameters of the environment and the reproductive cycle of the animal? Does the accumulation of these TE in *A. noae* soft tissue induce oxidative stress and disruption of the cell defense balance in these organisms?

In general, what is the state of health of *A. noae* in the Bizerte lagoon and possible consumption of this marine product doses poses risks to human health?

Material and methods

Study area

Bizerte lagoon is located on the northern coast of Tunisia (37° 11′ 20.4″ N 9° 51′ 16.2″ E) in the Western Mediterranean Basin

(Fig. 1). It covers an area of 128 km² and has a mean depth of 7 m, a maximum width of 11 km and a maximum length of 13 km. It is connected to the Mediterranean Sea through a 6-km-long channel and communicates with the Ichkeul Lake (110 km²) through the 5-km-long and 3-m-deep Tinja channel (Béjaoui et al. 2008). This lagoon is located near several towns (Bizerte, Zarzouna, Menzel Abderrahmen, Menzel Jemil and Menzel Bourguiba), industrial facilities and agricultural fields that affect the area. Bizerte lagoon is a particular ecosystem regarding its hydrodynamic and biogeochemistry (Béjaoui et al. 2008). Five principal streams called "wadis," Tinja, Rharek, Ben Hassine, Haima and Guenniche, feed the lagoon non-permanently with freshwaters depending mainly on rainfall. The water supply of the "Rharek wadi" (Fig. 1), the nearest wadi to the sampling site, is characterized by a restricted catchment area (8 km²) and a low runoff coefficient (0.66 ± 0.08 mm³/year). Nevertheless, the fairly high annual solid yield of this wadi ($1.46 \pm 0.32 \times 10^3$ T/year) results in very turbid waters inflows (Ben Garali 2009).

For this study, we monitored the southern less anthropized area of the lagoon. The sampling site (37°09′03″N 9°52′03″E) was far from urban and industrial sources of contamination but remained influenced by agricultural inputs (Barhoumi et al. 2014; Mahmoud et al. 2010).

Animal sampling and processing

A total of 220 sexually mature *A. noae* of commercial size (~50 mm) were collected the 15th of each month by Scuba Diving at about 3 to 5 m depth from October 2013 to August 2014. Samples were carried to the laboratory in a cool box. The shell length (SL), height (SH) and width (SW) were measured with a digital caliper to the nearest 0.1 mm. Regardless of sex, for TE analysis, *A. noae* ($n = 10$ bivalves per month) were dissected and their whole body soft tissues stored at -20 °C until analysis. For biomarker analysis, specimens ($n = 10$ bivalves per month) were dissected and their individual whole body soft tissues were frozen quickly in liquid nitrogen and stored at -80 °C until analysis.

Environmental parameters

Temperature (°C), salinity (S) and pH of Bizerte lagoon subsurface water were recorded using a WTW-197i multimeter (inoLab®). Subsurface water samples (2 L) were collected in a polyethylene bottle and transported to the laboratory in a cool box. Environmental parameters recording and water sampling were performed monthly between 8 and 9 a.m. local time from October 2013 to August 2014 and were concomitant to *A.noae* collection. Water samples were analyzed for suspended particulate matter (SPM, in g/L) and chlorophyll *a* (Chl *a*, in mg/L) in the laboratory according to Aminot and Chaussepied (1983) with standard methods recommended for marine waters. Chlorophyll *a* was extracted after filtration on Whatman GF/F filters with 90% methanol and concentrations were determined spectrophotometrically at 665 and 750 nm. SPM dry weight was determined after filtration of 1 L of
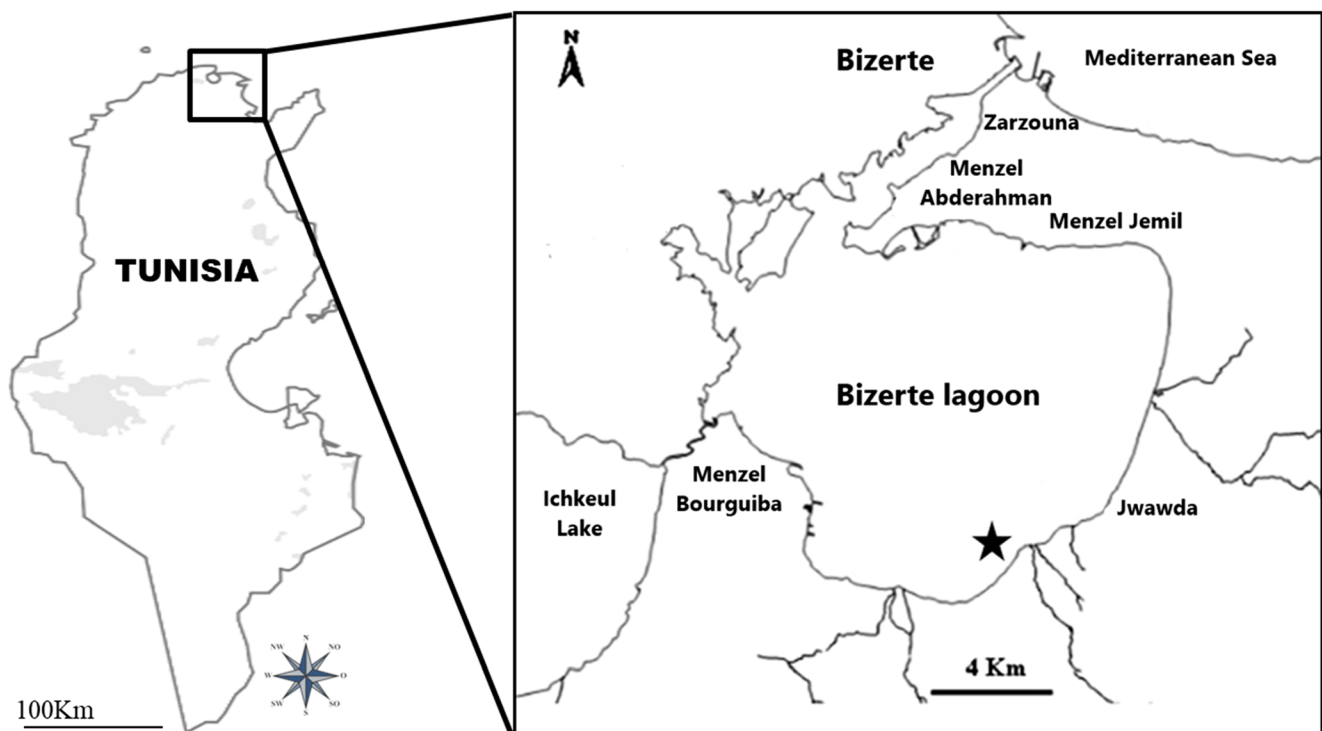

Fig. 1 Map of Bizerte lagoon (Northern Tunisia). The black full star in the Southern part of the lagoon shows the sampling site location

seawater. Precipitation data during the year 2013–2014 was provided by the Tunisian Institute of Meteorology.

Trace element analysis

Only materials in ceramic, plastic and glass were used for sample treatment and storage. Soft tissues were rinsed with Milli-Q water to remove extraneous material. Whole soft tissues of 10 mollusks per sampling date were lyophilized separately. Dried specimens were ground to powder using an agate mortar and pestle. The wet and dry weight (before and after the drying process) of the samples was determined to the nearest 0.001 g using an electronic precision balance (KERN: ABJ 120-4M). According to the method described in Richir and Gobert (2014), dried powders (0.3 g of dried sample) were mineralized in Teflon bombs in a closed microwave digestion labstation (Ethos D, Milestone Inc.) using 2 ml of nitric acid (HNO_3, 60%) and 1 ml of hydrogen peroxide (H2O2, 30%) as reagents (suprapur grade, Merck, Darmastadt, Germany). Digestates were diluted to an appropriate volume of 50 ml prior to being analyzed. The concentrations of essential elements (Zn, Fe, Cu Cr, Mn and Se) and non-essential elements (Al, Pb, Cd and Ni) were determined by inductively coupled plasma mass spectrometry (ICP-MS) using dynamic reaction cell (DRC) technology (ICP-MS ELAN DRCII, PerkinElmer Inc.). This instrument uses ion-molecule reactions to overcome spectral overlaps and requires selection of the appropriate reaction gas (Olesik and Jones, 2006): no reaction gas in standard mode (for ^{111}Cd and ^{208}Pb), NH3 (for ^{27}Al, ^{52}Cr, ^{54}Fe, ^{55}Mn, ^{60}Ni, ^{63}Cu and ^{66}Zn) or H2 (for ^{78}Se) in DRC modes. The purity of the chemicals used was verified by running several chemical blanks and no evidence of contamination was found. Analytical accuracy was checked by analyzing Certified Reference Materials: NIST 1566b (oyster tissue; mean recovery = $95 \pm 8\%$) and NIST 2976 (mussel tissue; mean recovery = $106 \pm 7\%$) from the American National Institute of Standards and Technology. The results obtained on the Certified Reference Materials were consistent with the certified values for all TE. For each element, detection limit (LD) and quantification limit (LQ) were calculated according to (Grinzaid et al. 1977; IUPAC et al. 1998; Currie, 1999), depending on their specific blank distribution. The trace element detection limits in mg kg^{-1} dry weight (DW) were as follows: Ni = 0.0142; Cr = 0.0030; Cd = 0.0005; Fe = 0.0341; Zn = 0.0504; Mn = 0.0107; Al = 0.0808; Cu = 0.0077; Se = 0.0423; Pb = 0.0025. The limit of quantification (LQ) for each metal was also calculated (Ni = 0.0468; Cr = 0.0099; Cd = 0.0016; Fe = 0.1125; Zn = 0.1663; Mn = 0.0353; Al = 0.2664; Cu = 0.0254; Se = 0.1395; Pb = 0.0082). Concentrations were analyzed as TE concentrations on a dry weight basis (mg/kg $_{DW}$) and averaged by seasons: autumn (October–November 2013), winter (December 2013 to February 2014), spring (March 2014 to May 2014) and summer (June 2014 to August 2014).

Biomarker analysis

Each whole animal soft tissue ($n = 10$) was thawed on ice and homogenized with an ultra Turrax® in phosphate buffer (0.1 M, pH = 7.4), then centrifuged at $9000 \times g$ for 20 min at 4 °C. Tissues homogenates aliquots were used for biomarker assays (malondialdehyde, glutathione peroxidase, reduced glutathione, metallothioneins and acetylcholinesterase). Biomarkers data were averaged according to the same seasons as for TE.

Protein quantification

Protein content (mg/g tissue) was determined in the whole soft tissue of *A. noae* according to Lowry et al. (1951), using bovine serum albumin (BSA) as standard (0.5 mg/ml). The protein content was used to quantify all biomarker responses.

Malondialdehyde

Malondialdehyde (MDA) level was determined spectrophotometrically according to Draper and Hadley (1990). Shortly, an aliquot of 0.5 ml of soft tissue extract supernatant was blended with 1 ml of trichloroacetic acid (30%). After centrifugation at $3500 \times g$ for 10 min, 1 ml of thiobarbituric acid reagent (0.67%) was added to 1 ml of supernatant and heated at 100 °C for 10 min. The mixture was then measured for absorbance at 532 nm. The MDA values were calculated using 1,1,3,3-tetraethoxypropane as standard and expressed as nmoles/mg protein.

Glutathione peroxidase

Glutathione peroxidase (GPx) activity in the whole soft tissues was measured according to Flohe et al. (1984). About 200 μl glutathione GSH (4 mM) was incubated with H_2O_2 (5 mM) and determined using Ellman's reagent (DTNB: 5,5-dithiobis-2 nitrobenzoic acid). The absorbance was measured at 412 nm. This reaction is carried out by the presence of GSH as a substrate. As a result, the activity of GPx was expressed as nmoles of GSH/min/mg protein.

Reduced glutathione

Reduced glutathione (GSH) was quantified by the method of Ellman (1959). A volume of 500 μl of homogenate was added to 3 ml of sulfo-salicylic acid (4%). The mixture was then centrifuged at $1600 \times g$ for 15 min. An aliquot of 500 μl of supernatant was taken and added to DTNB. The absorbance

was measured at 412 nm after 10 min. Total GSH was expressed as µg/mg protein.

Metallothioneins

Metallothioneins (MTs) were estimated according to the spectrophotometric method of Viarengo et al. (1997) modified by Petrovic et al. (2001). An aliquot of 500 µl of supernatant was mixed with absolute ethanol (500 µl) and chloroform (40 µl), followed by centrifugation at 6000×*g* for 10 min. After the addition of ethanol and incubation for 1 h at 4 °C, pellets were washed with ethanol (87%) and chloroform (1%), then centrifuged for 10 min. Pellets were suspended in NaCl (0.25 M) and EDTA (1 mM). DTNB (0.6 mM) dissolved in phosphate buffer (pH = 8) was added to the sample. The absorbance was measured at 412 nm. MTs concentration was expressed as µmoles of GSH/g protein.

Acetylcholinesterase activity

Acetylcholinesterase (AChE) activity was measured in the whole soft tissues according to the method of Ellman et al. (1961) using acetylthiocholine iodide as a substrate, phosphate buffer (0.1 M; pH = 7.4) with DTNB (0.01 M). Enzyme activity was recorded over 5 min after addition of the substrate. AChE activity was expressed as nmoles/min/mg protein.

Human risk assessment analysis

Estimated weekly intake

The daily intake of TE depends on both their concentrations in food and daily food consumption. In addition, the consumer body weight can influence the tolerance of contaminants (Chamannejadian et al. 2013). In this work, risk of TE intake was estimated by calculating the respective levels measured in *A. noae*, for a person weighing 60 kg and with a consumption rate of 154 g per week (a medium level mussel consumer, see Jovic and Stankovic 2014). The estimated weekly intake of each TE was determined using the following Eq. (1):

$$\text{Estimated weekly intake : EWI} = (C_m \times IR_w)/BW \qquad (1)$$

where C_m is the TE concentration in *A. noae* soft tissue (mg/kg $_{WW}$), IR_w the weekly ingestion rate (kg) and BW the body weight (kg).

To assess public health risks, weekly Ark shell intakes were compared with the Provisional Tolerable Weekly Intake (PTWI) recommended by the joint FAO/WHO Expert Committee on Food Additives (FAO/WHO, 2011).

Target hazard quotient

The target hazard quotient (THQ) of a contaminant, defined as the ratio between the exposure and the reference dose (RfD) for that contaminant, expresses the risk of non-carcinogenic effects (Yi et al. 2011). If the ratio is less than 1, there will be no obvious risk (Chien et al. 2002). Conversely, an exposed population will experience health risks if the exposure dose is equal to or greater than the RfD, so the ratio above 1 (Marengo et al. 2018). Since the exposure to two or more contaminants may result in additive or interactive effects, we evaluated the total THQ (TTHQ) by summing THQ value of individual TE. The models for estimating THQ and TTHQ follow Eqs. (2) and (3):

$$THQ = (EF \times ED \times IR_d \times C)/(RfD \times BW \times AT) \qquad (2)$$

$$TTHQ = THQ\,(TE1) + THQ\,(TE\,2)\ldots + THQ\,(TE\,n) \qquad (3)$$

where EF is the exposure frequency (365 days/year), ED is the exposure duration (70 years, average lifetime), IR_d is the food ingestion rate (0.0022 kg per day per person; Jovic and Stankovic 2014), RfD is the oral reference dose (Marengo et al. 2018; Nadal et al. 2008), BW is the average adult body weight (60 kg) and AT is the average exposure time for non-carcinogens (365 days per year x ED, assuming 70 years).

Target cancer risk

Target cancer risk (TR) indicates an incremental probability of an individual developing cancer over a lifetime due to exposure to a potential carcinogen (Saha et al. 2016).TR, derived from the intake of Pb, was calculated since this element may promote both non-carcinogenic and carcinogenic effects depending on the exposure dose (Vieira et al. 2011). Cancer risk over a lifetime exposure to Pb was obtained using cancer slope factor (CPSo) provided by the USEPA (USEPA 2010). The model for estimating cancer risk follows Eq. (4):

$$TR = (EF \times ED \times IR_d \times C \times CPSo)/(BW \times AT) \qquad (4)$$

where CPSo is the oral carcinogenic slope factor of 0.0085 (mg/kg/day)$^{-1}$ for Pb from the Integrated Risk Information System (USEPA 2010) database.

Acceptable lifetime cancer risk levels range from 10^{-4} (indicating a probability of 1 in 10,000 chance of developing cancer) to 10^{-6} (indicating a probability of 1 in 1,000,000 chance of developing cancer). The four TR categories are as follows: $TR \leq 10^{-6}$ = low, 10^{-4} to 10^{-3} = moderate, 10^{-3} to 10^{-1} = high and $\geq 10^{-1}$ = very high (Marengo et al. 2018).

Statistical analysis

Data were analyzed using the software STATISTICA 8 (StatSoft Inc.) and GraphPad Prism 5 (GraphPad Software Inc.).

The significance level was 0.05 for all statistical analyses. After testing for normality (Shapiro–Wilcoxon test) and homoscedasticity (Levene's test), significant differences between seasons for TE and biomarkers were determined with one-way analysis of variance (ANOVA) followed by a post-hoc Tukey's test. If conditions for ANOVA were not fulfilled, non-parametric Kruskal–Wallis's test was used. Correlations between biochemical parameters, essential elements (Zn, Fe, Cu Cr, Mn and Se) and non-essential elements (Al, Pb, Cd and Ni) and biomarkers, were studied using non-parametric Spearman's rank correlation. Principal components analysis (PCA) was performed on a data matrix with seasons as objects and TE, biomarkers (MDA, GPx, GSH, MTs and AChE), gonad index (GI) and environmental parameters (T, S, pH, Chl a and SPM) as variables.

Results

Shell biometry and environmental parameters

During the study period, the shell length of *A. noae* ranged from 54.4 ± 1.8 to 56.0 ± 0.5 mm, the shell height from 29.0 ± 1.3 to 31.2 ± 0.4 mm and the shell width from 26.3 ± 3.0 to 28.3 ± 0.4 mm (Table S1). Seasonal variations in water temperature (°C), salinity, pH, Chl a (mg/L), SPM (g/L) and rainfall (mm) are presented in Table 1. Subsurface water temperature (T) ranged from a minimum of 13.00 ± 1.00 (°C) in winter to a maximum of 27.33 ± 1.15 (°C) in summer. Salinity (S) varied from 34.57 ± 1.32 in winter to 38.20 ± 0.62 in summer. Rainfall ranged from 1.07 ± 1.15 (mm) in summer to 91.13 ± 61.83 (mm) in winter. Two important peaks of Chl a were measured during winter (0.77 ± 0.81 mg/L) and spring (1.41 ± 0.95 mg/L). As for the seasonal variation of SPM, two peaks were also observed, the first one in winter (0.05 ± 0.02 g/L) and the second one in spring (0.04 ± 0.01 g/L). pH values remained relatively constant during our sampling period, ranging from 8.05 ± 0.07 in autumn to 8.57 ± 0.15 in summer. Significant positive correlations ($p < 0.05$) were observed between temperature and salinity (rho = 0.876), between pH and both temperature (rho = 0.680) and salinity (rho = 0.640) and between Chl a and both temperature (rho = 0.270) and pH (rho = 0.250). SPM was significantly negatively correlated to T (rho = − 0.427), S (rho = − 0.575) and pH (rho = − 0.449) (Table 2).

Trace element concentrations in *A. noae* soft tissue

Mean seasonal TE levels (mg/kg $_{DW}$) determined in the whole soft tissue of *A. noae* are given in Table 3. TE concentrations in *A. noae* soft tissue decreased in the following order: Zn > Fe > Al > Mn > Cu > Se > Pb > Cd > Ni > Cr. *A. noae* soft tissue contained more essential elements (e.g., Zn and Fe) than toxic elements (e.g., Pb and Cd). Zn concentrations were stable during seasons (149.76 ± 18.09 to 172.45 ± 13.91 mg/kg $_{DW}$, $p > 0.001$). In contrast, concentrations of Cu, Cd and Ni significantly differed ($p < 0.001$) between seasons, with minimal values recorded during autumn-winter (3.78 ± 0.16; 0.57 ± 0.01 and 0.35 ± 0.03 mg/kg $_{DW}$, respectively) and maximal values during summer (6.37 ± 0.40, 0.91 ± 0.22 and 0.59 ± 0.12 mg/kg $_{DW}$, respectively) (Table 3). Fe ranged from 128.41 ± 22.97 (mg/kg $_{DW}$) during winter and 182.91 ± 40.94 (mg/kg $_{DW}$) during summer ($p < 0.001$). Same trends were observed for Al, Mn, Pb and Se concentrations ($p < 0.001$) with minimal values detected during winter (20.83 ± 3.14; 11.41 ± 5.34; 0.49 ± 0.09 and 3.32 ± 0.37 mg/kg $_{DW}$, respectively) and maximal values during summer (117.02 ± 38.92; 19.60 ± 5.75; 1.83 ± 1.68; 4.74 ± 0.14 mg/kg $_{DW}$, respectively). High levels of Cr were also observed during summer (0.38 ± 0.16 mg/kg $_{DW}$; $p < 0.001$) (Table 3).

Biomarker responses

Malondialdehyde levels

MDA levels differed significantly ($p < 0.001$) between summer and the other season, from a minimum of 10.43 ± 1.32 nmoles/mg protein in winter to a maximum of 33.73 ± 9.25 nmoles/mg protein in summer, respectively (Fig. 2a).

Glutathione peroxidase activity

GPx activity increased seasonally and significantly ($p < 0.001$) from spring to summer, from a minimum of 2.25 ± 0.39 nmoles GSH/min/mg protein to a maximum of 7.25 ± 2.21 nmoles GSH min/mg protein (Fig. 2b).

Table 1 Seasonal variation of water parameters (mean ± SD) of the studied site in the Southern part of Bizerte lagoon

	Temperature (°C)	Salinity	Chlorophyll a (mg/L)	SPM (g/L)	pH	Rainfall (mm)
Autumn 2013	20.00 ± 2.83	36.25 ± 0.35	0.63 ± 0.28	0.03 ± 0.01	8.05 ± 0.07	58.15 ± 14.07
Winter 2014	13.00 ± 1.00	34.57 ± 1.32	0.77 ± 0.81	0.05 ± 0.02	8.10 ± 0.10	91.13 ± 61.83
Spring 2014	17.33 ± 3.06	35.27 ± 0.49	1.41 ± 0.95	0.04 ± 0.01	8.23 ± 0.06	57.27 ± 49.64
Summer 2014	27.33 ± 1.15	38.20 ± 0.62	0.57 ± 0.28	0.02 ± 0.00	8.57 ± 0.15	1.07 ± 1.15

Table 2 Correlation matrix of non-parametric Spearman's rank correlation coefficients between *A. noae* gonadal index (GI), biomarkers (AChE, GPx, GSH, MDA, MTs), trace element soft tissue levels (Ni, Fe, Cr, Mn, Cu, Zn, Cd, Al, Se, Pb) and environmental (T, S, pH, SPM, Chl *a*) parameters measured in this study*e*

	Ni	Fe	Cr	Mn	Cu	Zn	Cd	Al	Se	Pb	AChE	GPx	GSH	MDA	MTs	S	T	pH	Chl*a*	SPM	GI
Ni	1																				
Fe	0.442	1																			
Cr	0.530	0.678	1																		
Mn	0.721	0.441	0.448	1																	
Cu	0.522	0.197	0.250	0.490	1																
Zn	0.237	−0.070	0.171	0.183	0.188	1															
Cd	0.397	0.218	0.359	0.232	0.245	0.237	1														
Al	0.436	0.778	0.631	0.361	0.275	−0.001	0.104	1													
Se	0.596	0.277	0.278	0.507	0.685	0.162	0.392	0.296	1												
Pb	0.376	0.096	0.072	0.472	0.623	0.109	0.160	0.197	0.421	1											
AChE	−0.460	−0.290	−0.299	−0.455	−0.260	−0.029	−0.188	−0.358	−0.445	−0.248	1										
GPx	−0.039	0.167	−0.019	0.156	0.056	−0.177	−0.126	0.102	−0.022	0.244	−0.024	1									
GSH	0.149	0.221	0.213	0.255	0.386	0.043	0.006	0.355	0.253	0.365	−0.022	0.311	1								
MDA	0.166	0.173	0.217	0.289	0.461	−0.023	0.031	0.286	0.237	0.545	−0.106	0.349	0.651	1							
MTs	0.490	0.264	0.329	0.303	0.419	0.059	0.435	0.320	0.605	0.328	−0.399	0.027	0.264	0.169	1						
S	0.257	0.373	0.230	0.421	0.483	0.076	−0.019	0.441	0.239	0.382	−0.302	0.460	0.614	0.474	0.195	1					
T	0.413	0.259	0.217	0.535	0.675	0.116	0.054	0.454	0.384	0.655	−0.347	0.277	0.634	0.610	0.307	0.876	1				
pH	0.511	0.224	0.322	0.386	0.610	0.174	0.186	0.316	0.548	0.294	−0.485	0.028	0.409	0.332	0.651	0.640	0.680	1			
Chl*a*	0.093	−0.158	−0.266	0.054	0.222	0.025	−0.230	−0.050	0.011	0.130	−0.224	−0.194	−0.277	−0.111	−0.062	0.126	0.250	0.270	1		
SPM	−0.243	−0.401	−0.139	−0.263	−0.249	0.160	−0.026	−0.403	−0.330	−0.257	0.496	−0.426	−0.442	−0.282	−0.479	−0.575	−0.427	−0.494	0.164	1	
GI	0.563	0.062	0.154	0.435	0.685	−0.027	0.079	0.230	0.548	0.458	−0.351	−0.100	0.228	0.402	0.373	0.503	0.685	0.665	0.317	−0.237	1

Numbers in italics indicate significant correlations at $p < 0.05$.

Table 3 Seasonal variation of trace element concentrations (mean ± SD; in mg/kg $_{DW}$; $n = 30$) in soft tissue of *Arca noae* sampled from Bizerte Lagoon

Seasons	Ni	Fe	Cr	Mn	Cu	Zn	Cd	Al	Se	Pb
Autumn 2013	0.35 ± 0.03[a]	147.25 ± 34.76[ab]	0.27 ± 0.12[a]	12.11 ± 4.60[a]	4.22 ± 0.77[a]	149.76 ± 18.09[a]	0.57 ± 0.01[a]	74.97 ± 18.56[b]	3.32 ± 0.37[a]	0.97 ± 0.44[a]
Winter 2014	0.39 ± 0.13[a]	128.41 ± 22.97[a]	0.26 ± 0.04[a]	11.41 ± 5.34[a]	3.78 ± 0.16[a]	168.06 ± 21.29[a]	0.77 ± 0.21[a]	20.83 ± 3.14[a]	3.55 ± 0.38[a]	0.49 ± 0.09[a]
Spring 2014	0.49 ± 0.12[a]	117.78 ± 13.95[a]	0.24 ± 0.06[a]	11.98 ± 3.51[a]	5.03 ± 0.72[a]	168.94 ± 18.45[a]	0.79 ± 0.08[a]	30.09 ± 8.02[a]	4.59 ± 0.35[ab]	1.13 ± 1.07[ab]
Summer 2014	0.59 ± 0.12[b]	182.91 ± 40.94[b]	0.38 ± 0.16[a]	19.60 ± 5.75[b]	6.37 ± 0.40[b]	172.45 ± 13.91[a]	0.91 ± 0.22[b]	117.02 ± 38.92[c]	4.74 ± 0.14[b]	1.83 ± 1.68[b]

Letters indicate significant differences at $p < 0.05$

Reduced glutathione levels

GSH levels differed significantly ($p < 0.001$) between summer and the other season (Fig. 2c). The lowest values were recorded in winter (1.21 ± 0.44 μg/mg protein) and the highest in summer (5.15 ± 2.62 μg/mg protein).

Metallothionein levels

MTs levels increased seasonally and significantly ($p < 0.001$) from autumn to summer, from a minimum of 3.58 ± 1.73 μmoles of GSH/g protein to a maximum of 10.72 ± 1.23 μmoles of GSH/g protein (Fig. 2d).

Acetylcholinesterase activity

AChE activity, as a neurotoxicity marker, ranged from 0.51 ± 0.05 nmoles/min/mg protein in autumn to 0.18 ± 0.10 nmoles/min/mg protein in summer, respectively. AChE activity seasonal decreased significantly ($p < 0.001$) from autumn-winter to summer season (Fig. 2e).

Human risk assessment analysis

The results of the human risk assessment analysis are presented in Table 4. EWI values by seasons are compared with the recommend PTWI. EWI of Cd, Cr, Cu, Fe, Pb and Zn by a 60-kg adult consuming 154 g of *A. noae* per week all over the year were all below the limit set by European regulations. THQ and TTHQ values for *A. noae* from Bizerte lagoon were lower than 1. TR of Pb varied from 8.8×10^{-7} to 1.43×10^{-6} and did not exceed the cancer risk.

Principal component analysis

The first two factorial axes of PCA explained 67.82% of the total variance of the data set with TE levels, biomarkers measured in *A. noae* and environmental parameters (Fig. 3a). More precisely, the 1st factorial axis (Factor 1) explained 51.09% of the total variance with Ni, Cr, Cu, Al, GSH, MTs, S, T and pH contributing positively to this one. Pb, GPx and MDA levels showed the highest weights in the 2nd factorial axis (Factor 2) that explained 16.73% of the total variance. The projection of seasons on the same factorial plane (1:2) showed a clear separation between summer and the other seasons. It was characterized by the highest values of all TE, oxidative stress biomarkers and abiotic parameters (T, S and pH). It coincides also with the second phase of *A. noae* reproduction and the increase of gonad index (GI). While, autumn, winter and spring seasons were characterized by the highest AChE activity and the abundance of SPM and Chl *a* levels in the lagoon waters (Fig. 3b).

Discussion

This study investigated for the first time the concentrations of TE and biomarker responses in the Ark shell *A. noae* collected seasonally from Southern Mediterranean coasts (Bizerte lagoon, Tunisia). Our scientific approach relied on multiple variables: TE, biomarkers of oxidative stress and environmental parameters at different periods of the species reproductive cycle. This study further assessed the risk linked to the consumption of this shellfish product by local populations.

Seasonal monitoring of TE levels in *A. noae* soft tissue showed significant differences ($p < 0.05$), revealing a large accumulation of essential TE than the toxic ones (Pb and Cd). This trend could be justified either by the fact that essential elements like Zn and Fe are fundamental for most living organism's physiological processes (spermatogenesis, enzymatic activity etc.) (Pinta 1980; Yamaguchi et al. 2009) or either by their bioavailability in the surface sediments of Bizerte lagoon as reported by Zaaboub et al. (2015) and Brik et al. (2018). Positive and significant correlations ($p < 0.05$) between TE concentrations in *A. noae* were observed (Table 2). These correlations probably reflected their similar bioaccumulation indicating a common source of inputs, derived from the ubiquitous enhances of human activities in the neighboring region. Indeed, interactions in TE bioaccumulation models can occur when they are in a mixture as in wastewater effluents or other sources of releases (George et al. 2013). This inter-elemental relationship for essential and non-essential elements accumulated in *A. noae* indicates strongly a similar pathway of metal uptake. TE levels in *A. noae* soft tissue were significantly higher in summer compared with other seasons ($p < 0.05$). This increase in summer could be due to the possible increase of TE concentrations in the lagoon waters, by runoff from adjacent farmland since *A. noae* sampling site is located near the agricultural area of Bizerte city. However, this hypothesis is rejected given the drought and the scarcity of rain during summer. Data precipitation showed a lack of rain during summer 2014 in Bizerte (see Table 1). Therefore, we suppose that the increase of TE levels in *A. noae* tissues in summer resulted from the evaporation process of Bizerte lagoon surface water. According to Béjaoui et al. (2017), water evaporation in Bizerte lagoon increases from 6.8 Mm^3 in winter to about 35.8 Mm^3 in summer. It has been previously reported that water evaporation and the increase of salinity could strongly lead to TE accumulation in bivalves during summer season (Staines-Urías et al. 2009; Strižak et al. 2014; Edokpayi et al. 2016). It has been shown in several studies that concentrations of the majority of TE emissions from anthropogenic activities accumulate in the river and oceanic sediments (Adamo et al. 2005; Bejaoui et al. 2018). The high evaporation rate might contribute to the concentration of TE in the water column, their deposition on the surface sediments and consequently on the bivalves tissues

(Bejaoui et al. 2018). Indeed, benthic invertebrates can absorb metals directly from sediments, either by absorption/adsorption from interstitial water or by direct ingestion, which will potentially affect other species through the food chain (Adamo et al. 2005; Chen et al. 2007). TE released from sediments via natural or anthropogenic processes will have harmful effects on the health of live species in these ecosystems (Phuong, 2014).

According to Frías-Espericueta et al. (1999), the main factors influencing TE levels in bivalves are the environmental factors (temperature, salinity, pH etc.), which affect their speciation (dissolved and/or particulate) in the water column. The accumulation of TE in marine organisms is largely influenced by speciation, i.e. the chemical form in which it exists (Amiard et al. 1987; Chiffoleau et al. 2001). In this study, most TE showed positive ($p < 0.05$) correlations with T, S and pH (Table 2). Temperature affects the chemistry of metals by modifying their speciation, solubility, reaction rates and/or physical kinetics (Blust et al. 1994; Byrne et al. 1988). Higher temperatures increase TE bioavailability and consequently their bioaccumulation, as reported by Rouane-Hacene et al. (2015) in *M. galloprovinciallis* from Algerian coasts. Salinity also appeared to influence the accumulation of TE in *A. noae* flesh from Bizerte lagoon. Phillips (1976) observed a net decrease in Pb accumulation in *Mytilus edulis* in low salinity conditions. In contrast, both temperature and salinity do not influence the bioaccumulation of certain trace elements, as it was reported in this study for Zn in *A. noae* soft tissue. Phillips (1976) reported that the accumulation of Zn in *M. edulis* tissues was not affected by seawater temperature and salinity. It also seems that pH affects the accumulation of TE in *A. noae* soft tissue. According to Basraoui et al. (2010), pH has a major influence on the speciation of TE in water as the bioavailability of TE increases when the environment is alkaline, while it decreases for low pH values (Pelletier, 2006). In general, physical, chemical and biological mechanisms interact and transform environmental contaminants into more or less available forms (Gourlay 2004). In addition, several authors have linked the increase in TE concentrations in the tissues of invertebrate and fish species during summer to the increase of metabolism because of high temperature (Ali and Abdel-Satar 2005; Ibrahim and Omar 2013). George et al. (2013) further explained that a substantial increase in the precipitated form of metals in the water column coupled with high filtration rate during the warm season could explain the high metal levels in bivalve tissues. Kamel et al. (2014) and Kefi et al. (2015) have also reported higher accumulation of Cd, Cu, Zn and Ni in *Mytilus galloprovincialis* from the Bizerte lagoon and for Pb in *Lithophaga lithophaga* in the Bizerte Bay during summer. Seasonal fluctuations in TE concentrations may be related to environmental conditions but also temporal variability in the physiological status of organisms (Bordin et al. 1992).

Fig. 2 Seasonal variations (mean ± SD; $n = 30$) of biomarker responses in the soft tissues of *A. noae* collected from Bizerte lagoon. **a** Malondialdehyde (MDA). **b** Glutathione peroxidase (GPx). **c** Reduced glutathione (GSH). **d** Metallothioneins (MTs). **e** Acetylcholinesterase (AChE). Letters indicate significant differences between seasons at $p < 0.05$
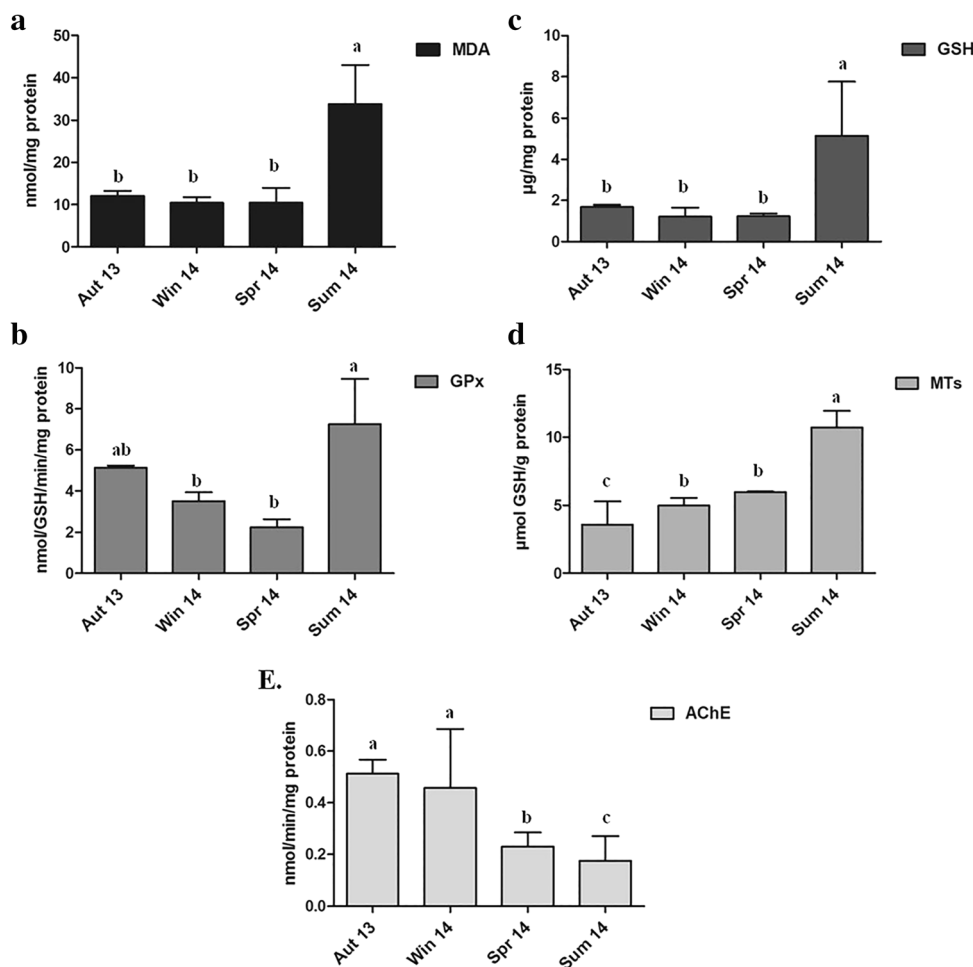
A. noae from the Bizerte lagoon shows a rather different pattern of reproduction when compared with *A. noae* populations from the Adriatic Sea. The reproductive cycle was characterized by two discrete maturing/spawning phases differing in terms of both length and intensity. The first one starts with a very rapid ovarian maturation phase in October to November, which is followed by a long, slow rate spawning phase extending from November to March. Afterward, a new ripening phase occurred from April to June/July, which is followed by a short massive spawning in late summer and early autumn (September) (Ghribi et al. 2017).

In our previous research, we reported that *A. noae* body dry weight (BDW) is relatively constant during the first reproductive phase (from October to March), while it increased significantly during the second phase from April to reach its maximum in July indicating a rapid increase in biomass caused by growth of gonadal and somatic tissues (see Ghribi et al. 2017). This increase coincided with high food uptake (Chl *a*) in the lagoon waters and important reserve materials accumulation (Ghribi et al. 2018). The use of the gonad index (GI), as a descriptor of the ecophysiological and health status of marine organisms, reflected in part the evolution of the sexual cycle of

A. noae in the Bizerte lagoon (Fig. S1). GI varied similarly to BDW confirming that gonadal mass mainly increased significantly during the second reproductive phase from early spring to reach its maximum in summer when gonads are fully ripe and gametes are ready to be released. Thereby, the high TE levels recorded in summer in *A. noae* soft tissue coincided with the significant increase in BDW and GI. GI was significantly positively correlated to five trace elements among all measured TE (Ni: $r = 0.563$; Mn: $r = 0.435$; Cu: $r = 0.685$; Se: $r = 0.548$ and Pb: $r = 0.458$ $p < 0.05$) (Table 2). Several studies have also reported higher TE concentrations in the flesh of several mollusk species during gonad maturation and lower ones during the spawning period (Cheggour et al., 1999; Idardare et al. 2011; Mubiana et al. 2005). Boussoufa et al. (2014) showed that the high bioaccumulation of Pb, Zn and Cu in *Donax truculus* from the Gulf of Tunis coincided with the period of gametogenesis and gonad maturation. However, this increase in TE levels in bivalve body soft tissues before spawning (maturation phase) is not systematic for all species. Many authors have reported a decrease in TE concentrations during this phase in *M. galloprovancialis* and *C. gigas* (e.g. Amiard and Berthet 1996; Raspor et al., 2004; Richir and

Table 4 Human risk assessment associated with the consumption of *Arca noae* harvested from the lagoon of Bizerte: trace element (TE), oral reference dose (RfD), provisional Tolerable Weekly Intake (PTWI), estimated weekly intake (EWI), target hazard quotient (THQ) and target cancer risk (TR)

TE	RfD	PTWI	EWI				THQ				TR			
			Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Cd	0.001	420	0.37	0.49	0.51	0.58	0.052	0.071	0.072	0.083				
Cr	0.003	38,220	0.17	0.17	0.15	0.24	0.008	0.008	0.007	0.012				
Cu	0.04	210,000	2.71	2.43	3.23	4.09	0.010	0.009	0.012	0.015				
Fe	0.7	336,000	94.33	82.13	75.72	117.43	0.019	0.017	0.015	0.024				
Mn	0.14						0.008	0.007	0.008	0.012				
Pb	0.00357	1500	0.62	0.31	0.73	1.17	0.022	0.011	0.026	0.042	7.558E−07	3.82E−07	8.805E−07	1.426E−06
Se	0.005						0.061	0.065	0.084	0.087				
Zn	0.3	420,000	96.25	107.80	108.44	112.93	0.046	0.051	0.052	0.054				
Total THQ							0.226	0.239	0.276	0.329				

Gobert 2014). This decrease is known as biological dilution results from the dilution of TE, when the volume of gonads increases during the maturation phase of gametes. Subsequently, TE are concentrated during egg-laying when the gonad volume decreases following the release of the gametes.

Comparing our results with those reported for *A. noae* from the Croatian (Cuculic´ et al. 2010, Ivankovic et al. 2016, Milun et al. 2016, Erk et al. 2018) and Yugoslav Adriatic coasts (Ozretic´ et al. 1990), Zn, Cu, Ni, Cr, Pb, Cd, Fe, Mn, Se and Al concentrations were within the same range of concentrations (Table 5). Concentrations of these TE in *A. noae* flesh were similar to lower when compared with other bivalves from Bizerte lagoon, Tunisian and Mediterranean coasts (Kamel et al. 2014; Richir and Gobert 2014; Boussoufa et al. 2014; Joksimovic et al. 2018; Prato et al. 2019; Uluturhan et al. 2019) (Table 5).

As producers of reactive oxygen species (ROS), TE accumulation and environmental factors could contribute and generate severe biological responses, known as biomarkers, leading to irreversible damages in marine organisms (Nieto et al. 2010). Oxidative stress biomarkers in *A. noae* from Bizerte lagoon showed an important temporal variability. In this study, the higher and positive correlation of MDA level recorded during summer with Cr, Mn, Cu, Al, Se and Pb (Table 2) could be associated with the rise of environmental factors (T, S etc.) and TE concentrations in the lagoon waters. However, during winter, MDA decrease might be related to low temperatures and rainfall that contribute greatly to the dilution of TE of the water surface. According to Bouki et al. (2013) and Mahmoud et al. (2010) the highest levels of MDA were recorded in *M. galloprovincialis* and *Fulvia fragilis*, respectively, from the Bizerte lagoon as a response to environmental TE levels increase.

In the current study, significant and positive correlation of GSH levels was detected ($p < 0.05$) with Fe, Mn, Cu, Al, Se and Pb levels in *A. noae* flesh from Bizerte lagoon (Table 2).

These results coincide with the findings of Mahmoud et al. (2010) carried on *F. fragilis* collected from Bizerte lagoon. Similarly, Bejaoui et al. (2017) reported high GSH levels in *V. decussata* digestive glands as a response to metal contamination in Boughrara lagoon (Tunisia). The seasonal variation of GPx activity was higher during summer and presents a positive correlation with Pb ($p < 0.05$) (Table 2). This increase could be an adaptive response to eliminate the effect of hazardous ROS generated by environmental stressors (pollution, T and S) as reported by Carregosa et al. (2014) in three species of veneridae. Our results also corroborate with the findings of Orbea et al. (2002). These authors reported that oysters from the Biscay bay exhibited the highest GPx activity during summer than winter.

The inhibition of AChE activity was also evaluated in the soft tissues of *A. noae* revealing a significant decrease during the summer season when compared with the other seasons. Such results could be related to exposure to many other chemicals like TE, hydrocarbons and detergents (Elumalai et al. 2002). AChE is present in neuromuscular junctions and cholinergic synapses in the central nervous system and stops signal transmission by hydrolyzing acetylcholine (Ach), a neurotransmitter that drives nerve impulses through neuromuscular junctions (Bocquené et al., 1997; Lodish et al. 2000). AChE inhibition has been used for years as a marker of exposure to aquatic contaminants (mainly insecticides) (Bocquené et al., 1997). In our study, the significant ($p < 0.05$) decrease of AChE activity in *A. noae* harvested during summer was negatively correlated with all TE concentrations except Zn and Cd (Table 2). Similarly, previous reports of Dellali et al. (2001), Barhoumi et al. (2014) and Kamel et al. (2014) have described the decrease of AChE activity in oysters and mussels collected from Bizerte lagoon from the most affected region by agricultural activities and industrial releases.

Besides, MTs levels in *A. noae* flesh collected from Bizerte lagoon varied between seasons and reached high concentrations in summer. This increase was positively correlated ($p < 0.05$) with all TE except Zn (Table 2). Due to the biological reactivity of metallothionein (MT) in response to the bioaccumulation of metals, it has been suggested that these low molecular weight proteins are suitable molecular indicators of metal pollution (Roesijadi, 1994). These metal-binding proteins are believed to participate in the functions associated with the metabolism and detoxification of metals (Marie et al. 2006). MTs roles comprise: (i) the homeostatic regulation of essential metals, such as Zn or Cu; (ii) detoxification via the chelation of harmful TE (Cd or Hg); and (iii) the protection of tissues against various forms of oxidative injury (Marie et al. 2006). With regard to seasonal variations, our results are consistent with the findings of De Clercq (2016), Kamel et al. (2014) and Leinio and Lehtonen (2005). These authors reported high MTs levels during summer in *A. noae* from the Adriatic Sea, in *M. edulis* from the Baltic Sea and in *M. galloprovancialis* from Bizerte Lagoon, respectively. Moreover, Geffard et al. (2002) and Chandurvelan et al. (2015) showed that high levels of MTs measured in situ and under laboratory-controlled conditions in oyster and mussel tissues following metals exposure.

To sum up, the high biomarker responses during summer appear to be mainly related to the accumulation of TE in *A. noae* flesh that inducing oxidative stress in organisms leading to physiological disturbances and antioxidants activation (Verlecar et al. 2007; Bejaoui et al. 2018). In addition, high biomarker responses during the summer period may also be related to seasonal changes in environmental parameters (high temperature, low oxygen levels etc.), as reported by Cravo et al. (2012) for *R. decussatus* in a low contaminated lagoon

(Ria Farmosa, Portugal). In this regard, the temperature seems to be also a determinant factor inducing biomarker responses in *A. noae* from Bizerte lagoon. The temperature was positively correlated with MDA, GSH, MTs levels and GPx activity, while it was negatively correlated with AChE activity (Table 2). The effect of temperature on biomarker responses and enzymatic activities has already been reported by other authors in different bivalve species (Barhoumi et al. 2014; Dellali et al. 2001). It has also been suggested that salinity fluctuation may influence the ability of bivalves to face oxidative stress (Prevodnik et al. 2007). It can be concluded that relationships between various biomarker responses are an adaptive response of *A. noae* to counteract the adverse effects of ROS caused by TE bioaccumulation but also stressful environmental conditions as discussed below.

From a nutritional point of view, TE concentrations on a wet weight basis in *A. noae* from the lagoon of Bizerte were well below international food safety standards and the maximum allowable level in bivalve flesh set by the international organizations Food and Agriculture Organization of the United Nations (FAO, 1983), European Community (EC 2006) and US Food and Drug Administration (USFDA 2001). EWI values for *A. noae* at the four different seasons were far below the PTWI values. Therefore, the consumption of this bivalve does not pose sanitary problems. EWI values are in agreement with values given in previous studies for bivalves (Storelli and Marcotrigiano 2001; Yusa et al. 2008) and fishes (Marengo et al. 2018; Turkmen et al. 2010). THQ is a useful parameter to assess the risk associated with the intake of TE contaminated shellfish (Storelli 2008) and fishes (Copat et al. 2013). The acceptable guideline value for THQ is 1 (USEPA 2010). THQ values were less than 1 for *A. noae* analyzed in this study, indicating no potential carcinogenic
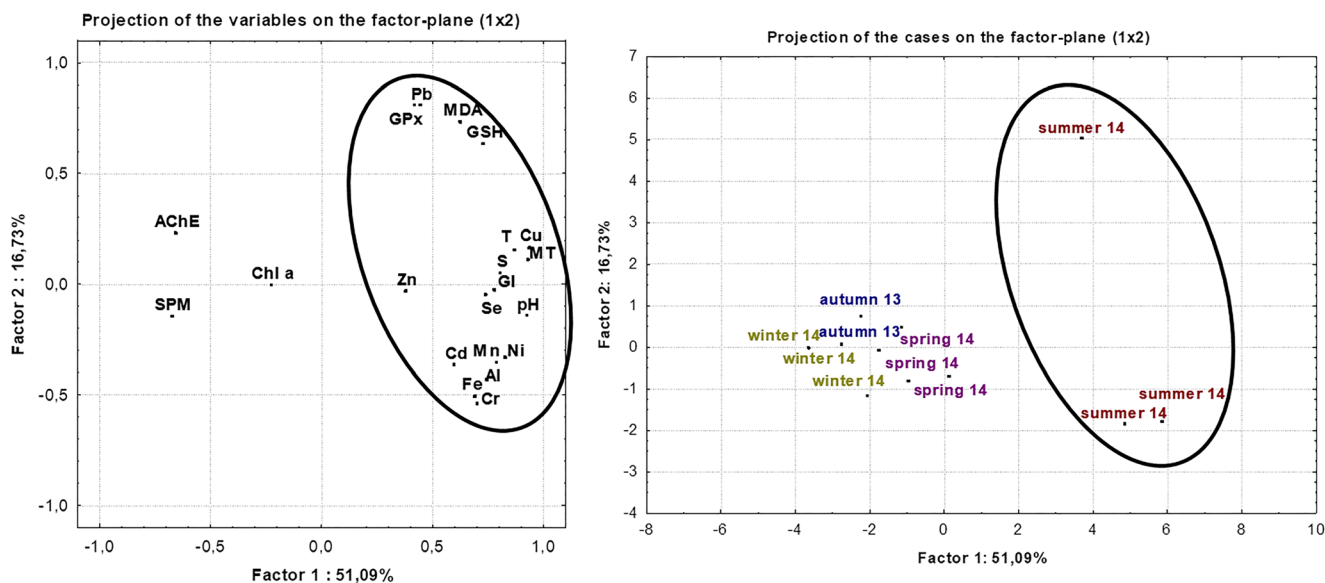

Fig. 3 Principal component analysis (PCA) of the examined TE, biomarker responses, body dry weight (BDW) and environmental parameters. (**a**) 2D factor loadings plot and (**b**) 2D scores plot in the space defined by the two first factorial axes of the PCA

Table 5 Comparison of TE concentrations (in mg/kg of dry weight (DW) or wet weight (WW)) in *A. noae* from Bizerte lagoon and the Adriatic Sea with other bivalves from different Mediterranean coastal areas. Concentrations are also compared with maximum levels of TE admissible in shellfish flesh, as set by the FAO, the EC and the USFDA

	Species	Location	Reference	Ni	Fe*	Cr	Mn*	Cu	Zn	Cd	Al*	Se*	Pb
1.	*Arca noae (DW)*	*Bizerte lagoon (Tunisia)*	*Present study*	*0.21–0.96*	*71.4–317*	*0.10–0.62*	*4.61–32.49*	*2.03–8.70*	*82.2–269*	*0.27–1.66*	*9.51–214.37*	*2.02–5.99*	*0.28–4.45*
	Arca noae (WW)			*0.05–0.24*	*17.58–79.25*	*0.02–0.15*	*1.15–8.12*	*0.50–2.17*	*20.55–69.75*	*0.06–0.41*	*2.37–53.5*	*0.50–1.49*	*0.07–1.11*
2.	*A. noae* (DW)	Adriatic Sea (Croatia)	Erk et al. (2018)	0.44–1.01	151–438	0.66–1.60	6.93–16.76	3.08–6.26	77–179	0.89–3.47	47–179	4.50–9.79	0.38–0.82
3.	*A. noae* (DW)	Adriatic Sea (Croatia)	Ivankovic et al. (2016)	0.45–1.14		0.47–1.70		3.08–6.82	100–358	0.68–3.67			0.31–1.20
4.	*A. noae* (DW)	Adriatic Sea (Croatia)	Milun et al. (2016)	0.95–4.64		0.87–3.43		3.69–202.3	55.76–4010.3	0.23–4.03			0.95–4.64
5.	*A. noae* (DW)	Adriatic Sea (Croatia)	Cuculic et al. (2010)					2.85–13.23	89.7–167.6	1.52–2.84			0.59–0.69
6.	*A. noae* (DW)	Adriatic Sea (Croatia)	Ozretic et al. (1990)							3.35			0.55
7.	*Mytilus galloprovancialis* (DW)	Bizerte lagoon (Tunisia)	Kamel et al. (2014)	1.68–4.16				1.72–3.70	22.84–43.17	0.18–0.49			
8.	*Donax trunculus* (DW)	Gulf of Tunis (Tunisia)	Boussoufa et al. (2014)			1.2–6	7–70	5.8–15.3	68.3–87.5	0.1–0.66			3.3–5.3
9.	*Modiolus barbatus* (WW)	Italian coast	Prato et al. (2019)	1.06–1.14		1.41–1.61				0.63–0.68			0.65–0.75
10.	*Tapes decussatus* (DW)	Turkish coast	Uluturhan et al. (2019)		257–1083	2.90–17.0	8.70–21.6	4.06–7.09	43.6–51.3	0.07–0.32			0.27–1.57
11.	*M. galloprovancialis* (DW)	Montenegrin coast	Joksimovic et al. (2018)			0.72–4.05		6.08–13.2	85.6–241	1.01–2.87			1.37–3.55
12.	*M. galloprovancialis* (DW)	Diane Pond (Eastern Corsica)	Richir and Gobert (2014)	0.71–3.39	66–656	0.19–2.17	3.4–20.6	2.8–7.4	35–224	0.21–1.03	39–877	1.5–4.3	0.14–1.10
13.	*M. galloprovancialis* (DW)	French Mediterranean coasts	RINBIO (2001)	2		1		2.9–9.2	116–203	0.1–5.9			0.5–5.4
14.	Bivalves (WW)		FAO (2003)					10–30	40–100	2			1–6
15.	Bivalves (WW)		EC (2006)							1			1.5
16.	Bivalves (WW)		USFDA (2001)	80		13				4			1.7

FAO Food and Agriculture Organization of the United Nations, *EC* European Communities, *USFDA* United States Food and Drug Administration

*No security limits defined for this element

risk from ingestion of a single TE through their consumption. Consumption of *A. noae* involved exposure to a mixture of TE. Values of cumulative health risks (TTHQ, the sum of health risks of the 10 TE) were however also less than 1, again indicating no potential significant health risk from TE ingestion. The risk of cancer due to exposure to Pb was not of concern since the values of TR were lower than the acceptable risk limit of 10^{-4} (USEPA 2010). From all these observations, it can be said that continued consumption over long periods of this shellfish may not represent a potential risk to the health of consumers regarding Zn, Fe, Al, Mn, Cu, Se, Pb, Cd, Ni and Cr. Our results for THQ, TTHQ and TR correspond to previous studies on fishes (Saha et al. 2016; Marengo et al. 2018; Vieira et al. 2011). In addition, we can estimate that the (sub) population of *A. noae* inhabiting the south part of Bizerte lagoon is potentially a safe food regarding TE and can be recommended for possible commercialization and human consumption in Tunisia. However, prior to that, the

investigation of *A. noae* potential contamination by other toxic compounds (OCPs, PCBs, HAPs etc.) is strongly recommended. If safety is observed for other chemicals as for TE, farming and exploitation of this species in Bizerte lagoon can be developed, after applying sanitary measures required to set out the basic rules for food safety and animal health standards.

Conclusions

The present study highlighted the seasonal accumulation of TE and environmental conditions on the metabolic responses of *A. noae*, in relationship with its reproductive cycle that was presented by the gonad index (GI). The battery of parameters applied in the present work including *A. noae* oxidative response (MDA, GPx, GSH, MTs and AChE) and TE accumulation provides a discrimination of seasons with different levels of contamination, especially during summer season. Regarding health risk assessment linked to *A. noae* consumption, all sanitary indicators (EWI, PTWI, THQ, TTHQ and TR) related to TE levels bioaccumulation were below the risk for human health standards. Consequently, *A. noae* can be considered as a safe shellfish product for human consumption in Tunisia, based on its beneficial properties for health and nutritional quality, so Tunisians are encouraged to consume this shellfish. This work is the first step towards the valorization of this unexploited shellfish species in Tunisia since this bivalve being already commercialized and consumed in other areas from the Mediterranean (Adriatic Sea). As perspectives for this study, other investigations must be determined using *A. noae* collected from other area. Additionally, studies on the TE levels in the sediment and waters are mandatory in order to present a total examination of the study area. Finally, we plan to confirm the correlations between field effects and measure exposure concentrations by controlled laboratory experiments."

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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