MORPHOMETRIC, REPRODUCTIVE AND ENVIRONMENTAL BIOMARKER ANALYSIS OF A CRUSTACEAN SPECIES *ATYAEPHYRA DESMARESTI* (MILLET, 1831) FROM NORTHEAST ALGERIA

AMEUR, A.[1] – BERGHICHE, H.[1*] – BAROUR, C.[2] – SOLTANI, N.[1]

[1]*Laboratory of Applied Animal Biology, Faculty of Sciences, Department of Biology, Badji Mokhtar University, 23000 Annaba, Algeria*

[2]*Laboratory of Aquatic and Terrestrial Ecosystems, Faculty of Natural and Life Sciences, Mohamed Chérif Messaadia University, Souk Ahras, Algeria*

[*]*Corresponding author*
e-mail: hindabentoubal@hotmail.ca; phone: +213-77-632-1543

(Received 18th Apr 2022; accepted 22nd Jul 2022)

Abstract. Comparative study was carried out on classical morphometric reproductive and biochemical parameters concerning populations of *Atyephyra desmaresti* sampled in northeast Algeria: the mouth of the Mafragh River with El Battah (site 1) is located in Annaba's Gulf, and Oubeira (site 2) and Tonga (site 3). Lakes are located in El Kala National Park. Principal component analysis revealed a very significant correlation between the morphometric characteristics of *A. desmaresti* in each site. Growth process seems relatively similar in the analyzed population except some abnormal values measured on the rostrum of individuals from site 2. The morphometric analysis also revealed a weak sexual dimorphism and higher abundance of *A. desmaresti* at site 2. The reproductive parameters analysis exhibited the following decreasing order in fecundity: site 3> site 1> site 2. As for egg diameter and vitelline (Vn) contents, the following decreasing order was observed: site 2> site 3> site 1. Biochemical analyses revealed significant differences in acetylcholinesterase (AChE) activity; moreover, a significant induction of glutathion S-transferase (GST) activity and a high significant level of metallothioneins (MTs) were recorded in *A. desmaresti.* These differences are discussed according to the characteristics of the studied sites. Data obtained could be useful in improving our knowledge of the Algerian shrimps.

Keywords: *comparative study, Annaba's Gulf, El Kala National Park, shrimps, biochemical analyses*

Introduction

The most productive ecosystems include marine, coastal, and wetland ecosystems. They include the open ocean, nearshore coastlines, places where freshwater and brackish are mixed, and inland wetlands. Wetlands play an important role in biodiversity and ecosystem function (Kumari et al., 2020), providing food sources and creating a livelihood for surrounding communities (Yetis and Akyuz, 2020). However, the flow of these ecosystem services can be altered by anthropogenic pressures.

In the last few years, natural water bodies have been in danger due to various pollutants (Landos et al., 2021). This chemical pollution involves many molecular families with different structures and functions, such as metal trace elements, polycyclic aromatic hydrocarbons polychlorinated biphenyls, plasticizers, petroleum derivatives, drug residues, solvents, pesticides and chemicals. Although some pollutants are not widespread and therefore will not cause acute toxic effects, many of these contaminants have bioaccumulation characteristics; they adversely affect aquatic ecosystems at all

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2006_45794597

trophic levels, from plankton to whales, biomagnify in the aquatic food web and eventually reach humans (Landos et al., 2021).

The species *Atyaephyra desmaresti* (Millet, 1831) is a small freshwater shrimp prawn, commonly known as Caridine. This species was first observed in North Africa and southern Europe, and usually found in the Mediterranean region. This species lives in aquatic vegetation, like rivers, canals, lowland areas, rich vegetation, weak water flow, and calcareous areas. It also occasionally occurs in estuaries and brackish waters. This shrimp is important in the trophic chain of the river ecosystem, as an intermediate link between plants and fish (Meurisse-Génin et al., 1985), and is characterized by its wide tolerance to salinity and temperature (Dhaouadi et al., 2006).

Quantification of morphometric parameters and bioenergetic reserves of marine invertebrates from different locations with a wide geographic range is necessary to assess the status of species populations, which can vary across spatial scales (Baldanzi et al., 2018). These variations can be modified by environmental parameters, geographic features, and ecological interactions. Multivariate morphometrics have been used successfully in population studies, stock discrimination, biogeographical studies and phenotypic plasticity (Von Cramon-Taubadeletal et al., 2005). It has been used in many studies of native decapods to compare geographically isolated populations, to show intraspecific variation (Kapiris and Thessalou-Legaki, 2001), and analyze the environmental interaction with species populations (Maynou and Sarda, 1997).

Female reproductive parameters are accurate indicators of the influence of environmental changes on organisms. Nevertheless, the reproductive performance of shrimp is influenced by many factors, including broodstock size and habitat (Cavalli et al., 1997), culture systems and environmental conditions (Otoshi et al., 2003), age and season (Crocos and Coman, 1997), diet (Lin and Shi, 2002), genetics and oxygen availability (Peruzza et al., 2018).

Biomarkers are often measured when an organism is exposed to pollutants and exhibits a cascade of biological responses triggered by stress (Gonçalves et al., 2020). They represent early warnings of alteration in population level that allows monitoring of environmental quality and specially to assess environmental changes (Lionetto et al., 2019). Among the most widely used biomarkers are biomarkers that indicate neurotoxicity, such as acetylcholinesterase (AChE) (Blaise et al., 2016), and biomarkers related to oxidative stress, like superoxide dismutase (SOD) (Farombi et al., 2007), catalase (CAT) (Bergayou et al., 2009), glutathione peroxidase (GSH. Px), glutathione S-transferase (GST), glutathione (GSH), glutathione reductase (GR) (Zhou et al., 2008). Malondialdehyde (MDA) an index of lipid peroxidation (Charissou et al., 2004) or metallothioneins (MTs) are widely used as a biomarker of metal contamination by binding and removing toxic metals (Pedrini-Martha et al., 2017).

The present research is carried out on *A. desmaresti* populations sampled at three sites: El Battah (in the mouth of Mafragh River) and Lakes of Tonga and Oubeira. It aimed firstly to assess the morphological variation among shrimps' populations by measuring (eight linear morphometric parameters) and (three weight parameters). Also, reproductive events of ovigerous females (fecundity, egg diameter) were analyzed. The second objective is to monitor the seasonal biochemical responses of some biomarkers (Vn, GST, AChE, MTs) to the environmental stress.

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DOI: http://dx.doi.org/10.15666/aeer/2006_45794597

Material and methods

Study area

The present work was carried out in the extreme northeast Algeria. For this, three sampling sites were selected and located using a Global Positioning Satellite (GPS). Site 1: El Battah (36° 50' 33.41"N, 07° 57' 9.89"E) is located at the level of the mouth of the Mafragh River on the coastal basin of El Battah. The estuary of the Mafragh River is formed at 1.8 km from the Mediterranean Sea. Site 2: Oubeira Lake (36° 51’ 52.83’’N, 08° 22’ 59.85’’E) is a freshwater shallow lake, located at 5 km from El Kala City (wilaya of El Tarf) this lake is of natural origin and covers an area of 2200 ha. Site 3: Tonga Lake (36° 53’ 1.03’’N, 08° 31’ 46.60’’E) is a freshwater wetland of 2600 ha connected to the Mediterranean Sea by the artificial strait of Messida. Although Tonga and Oubeira Lakes are located in El Kala National Park, according to Ramsar Convention, these two lakes constitute an important source of water supply (*Fig. 1*).

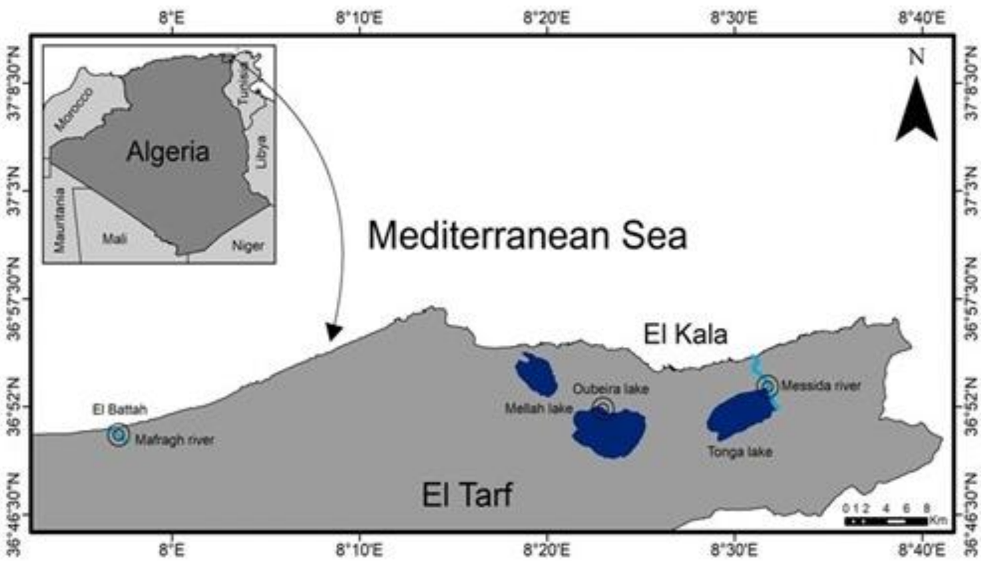

Figure 1. *Map showing the sampling sites of A. desmaresti in the extreme northeast of Algeria. Site 1: El Battah, site 2: Oubeira Lake, site 3: Tonga Lake*

Sampling procedure

Shrimps were collected using seine nets (length: 8 m; pocket: 1.60 m; mesh size 4 mm) during both spring and autumn seasons of two consecutive years (2019 and 2020) for the morphometric study. However, in spring 2021, only *A. desmaresti* females were selected to study reproductive parameters (*Table 1*). Specimens were found in aquatic vegetation where they find abundant and varied food consisting of diverse microfauna and microflora. In addition, water temperature, salinity, dissolved oxygen and pH were recorded using a multi-parameter water analyzer (HI 9829).

Morphometric measurements

Different morphometric parameters were recorded on each specimen after identification of species and sex determination that have been randomly sampled from the study sites (3099 shrimps). Sex identification in *A. desmaresti* was examined by

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microscopic inspection of the first pair of pleopods which carried the genital appendages of males (Dhaouadi et al., 2006). The total weight (TW), weight of the flesh (FW), weight of the cuticle (WC) of the shrimp were carried out using an Ohauss Type PA 214C Analytical Balance with a precision of 1/10 mg, while total length (TL), standard length (SL), cephalothoracic length (CL), abdominal length (AL), uropod length (UL), rostrum length (RL), telson length (Tel.L) and cephalic height (CH) were measured using a Tracaeble Fisher scientific caliper. The total number of eggs per ovigerous female (i.e., fecundity) was counted after the extraction of the egg masses from the pleopods of 120 shrimps (*Fig. 2A*). The diameter of the eggs was determined after dissection under a specialized microscope (Leica DM500) connected to a computer and the results were expressed in micrometers (*Fig. 2B*).

Table 1. *The number of A. desmaresti individuals by sex within each site used for both biometric and reproductive studies sampled during 2019-2021 period*

	Morphometric study				**Reproductive study**
	2019		**2020**		**2021**
Site	**Female**	**Male**	**Female**	**Male**	**Female**
El Battah	334	166	141	159	32
Oubeira Lake	472	227	344	156	68
Tonga Lake	417	283	247	153	20
Total	3099				120

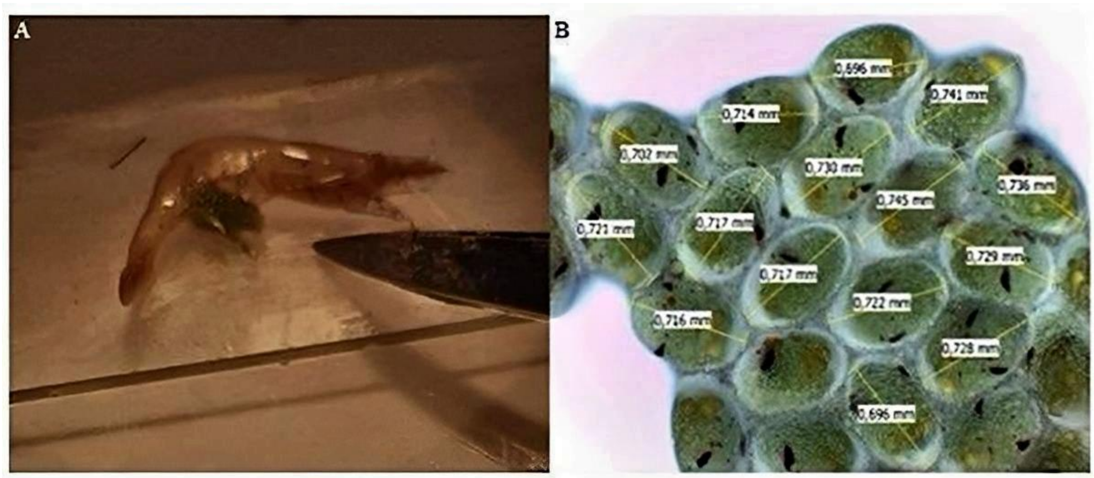

Figure 2. A: *Dissection of ovigerous female shrimp of A. desmaresti and collection of egg mass,* ***B:*** *Measurement of egg diameter under the Leica DM500 light microscope connected to a computer*

Biochemical procedure

Vitelline (Vn) quantification in eggs of some grained shrimps was made according to the procedure of Fabre et al. (1990) described by Cheghib et al. (2020), where eggs were placed in 500 µL of Tris-HCl-NaCl and homogenized. After centrifugation (5000 rpm for 10 min), three different layers are separated, the middle layer containing vitelline is removed and stored (-20 °C) until analysis.

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After dissection of *A. desmaresti* shrimps, flesh fragments (weight: 49-50 mg) were used for glutathion S-transferase (GST) measurements and heads were used for acetylcholinesterase (AChE) and metallothioneins (MTs) assays. GST activity was performed according to Habig et al. (1974) as previously described (Denna et al., 2022). Changes in absorbance were recorded at 340 nm with a spectrophotometer “Agilent Technologies” type CARY 60 UV-Vis. AChE activity was assessed following the procedure of Ellman et al. (1961) described elsewhere (Douafer et al., 2020) with the use of acetylthiocholine (ASCh) as substrate Measurements were conducted at a wavelength of 412 nm with a run time of 20 minutes. The activity of GST and AChE was expressed as μM/mn/mg protein. MTs were determined according to the method of Viarengo et al. (1997) recently described (Amira et al., 2018) using glutathione (Sigma) as a standard. The levels of MTs were calculated by evaluating the SH residue content using the Ellman’s reagent (DTNB: 5,5- dithiobis 2 ni-trobenzoic acid). Absorbances were yellow at 412 nm and metallothionein levels quantified using glutathione (Sigma) as a standard. The results were expressed as μg of MTs/mg of protein.

Results were expressed as g/mg protein. Protein concentrations in the total homogenate were also quantified according to the method of Bradford (1976) with Coomassie brilliant blue G250 (SigmaAldrich) as a reagent and bovine serum albumin (Sigma, Germany) as a standard. The absorbance was read at 595 nm. The tests were performed with 15-20 repeats for each season at each site.

Statistical analysis

All statistical analyses were performed in R (R Core Team, 2021) statistical language (Ihaka and Gentleman, 1996) version 4.1.2. for MacOS (https://cran.r-project.org). All data were expressed as mean ± standard error (SE). The normality of all variables data was tested by Shapiro-Wilk test. Comparison between studied sites and seasons were carried out to assess the variation of our studied parameters, using the non-parametric Kruskal-Wallis rank sum test followed by a non-parametric pairwise Dunn’s test with Bonferroni adjustment to find post-hoc statistical differences. Different lowercase letters indicate a significant difference between studied sites. Furthermore, to investigate a possible association between reproductive parameters we calculated then non-parametric Spearman’s rank correlation. The statistical analyses (tests) were applied at α= 0.05 as a significance level. We carried out also a multivariate analysis by applying a principal component analysis (PCA) to characterize the shrimps’ morphometric structure between sites, between seasons and among males and females. Moreover, we used in our statistical analyses many R packages: 'FactoMineR' (Lê et al., 2008), 'factoextra' (Kassambara and Mundt, 2020), 'ggplot2' (Wickham, 2016), 'Hmisc' (Harrell, 2021), 'ggcorrplot' (Kassambara, 2019) and 'dunn.test' (Dinno, 2017). The number of individuals tested per series is given with the results.

Results

Physico-chemical parameters of the water from the studied sites

The spatiotemporal variations of physicochemical parameters of the water are presented in *Table 2*. The recorded data shows maximum average values of temperature during autumn 2020, and a minimum value recorded in spring 2019 at site 1 (the mouth of the Mafragh River). The pH, in contrast, reaches a maximum value during the

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autumn of 2020 at site 2, and a minimum value in spring 2019 at site 3. The maximum of salinity is recorded at site 1 during spring 2020 and the minimum at site 2 during spring 2019. Dissolved oxygen reached a maximum value in autumn 2019 at site 3, and a minimum value at site 1 during spring 2020. So, increase in temperature was recorded in 2020 compared to the year 2019, and this is probably due to global warming and drought episodes that the world experienced that year (World Meteorological Organization – WMO –, April 2021).

Table 2. *Seasonal variations of physicochemical parameters of water during 2019-2020 period (m ± SD)*

Years	Site	Season							
		Temperature (°C)		**Potential Hydrogen (pH)**		**Salinity (PSU)**		**Dissolved oxygen (%)**	
		Spring	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**
2019	**El-Battah**	20.92±3.84	23.51± 3.82	7.6±0.83	7.85±0.56	5,80± 3.68	3.55±1.27	5.93±0.69	6.43±0.32
	Oubeira Lake	21.79±5.48	22.93±3.34	8.22±0.30	8.65±0.60	0.30±0.01	0.31±0.01	11.63±4.21	12.78±1.81
	Tonga Lake	22.50±3.23	22.94 ±4.63	7.53±0.09	7.57 ±0.08	0.43 ±0.16	0.38 ±0.10	12.65 ±1.90	23.5 ±0.40
2020	**El-Battah**	21.08±3.88	23.64 ±3.91	7.80 ±0.74	8.27 ±0.31	6.18 ±3.94	3.61 ±1.29	5.55 ±0.31	6.27 ±0.40
	Oubeira Lake	22.14±5.74	23.17±3.26	8.29±0.33	8.69±0.52	0.31±0.02	0.32±0.02	11.59±4.20	12.73 ±1.84
	Tonga Lake	22.56±3.16	23.20 ±4.85	7.59 ±0.28	7.83 ±0.11	0.45 ±0.24	0.40 ±0.17	12.63 ±1.67	23.17 ±0.31

The comparisons of the medians relating to the physicochemical parameters between our three sites revealed significant differences ($p < 0.05$) in pH, salinity and dissolved oxygen; while there was no significant difference for temperature variable ($p > 0.05$).

Morphometric measurements

For this reason, the first part of the study will assess the different morphometric (linear and weighted) and reproductive (fecundity and egg diameter) parameters in order to explain the spatiotemporal variations of growth and reproduction in *A. desmaresti* species. For this, a total of 3099 individuals of *A. desmaresti* were collected at the three study sites between 2019 and 2020.

Overall, PCA in relation to the shrimp growth data (linear and weight characteristics) showed a clear correlation between linear and weight measurements during the 2019-2020 period (*Fig. 3A*). In addition, the PCA resulted in two relevant components that accounted for 95.3% of data variance (total inertia). On the one hand, PC1 alone represented by 87.2% of the variance and showed strongly positive correlation especially with: TL (r= +0.99; cos^2= +0.98), SL (r= +0.99; cos^2= 0.98), AL (r= +0.99; cos^2= 0.98), and UL (r= +0.98; cos^2= 0.96). On the other hand, PC2 just explained 8.1% of the total data variance and it was also positively correlated with the RL (r= +0.75; cos^2= 0.56). Furthermore, the PC1 clearly indicates a morphometric variation within sites and it seems that *A. desmaresti* displayed a similar growth process in particular in El Battah (site 1) and Tonga (site 3) (*Fig. 3B*). Likewise, *A. desmaresti* species is characterized by a weak sexual dimorphism which is explained by the overlap of the two groups presenting females and males (*Fig. 3C*); and secondly, according to PC1 we noted, firstly that shrimps fished in autumn were larger than those fished in spring (*Fig. 3D*). By contrast, in Oubeira Lake (site 2) we noted some abnormal cases in the growth process, where some specimens are characterized by a greater length of rostrum

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RL contrary to those fished in the two other sites. This specific morphological difference is explained by the PC2 and could make a case for the effect of pollution on the shrimp growth physiology. The resultant ordination plots showed distinct partitioning of *A. desmaresti* samples according to site, sex and season factors (*Fig. 3*).

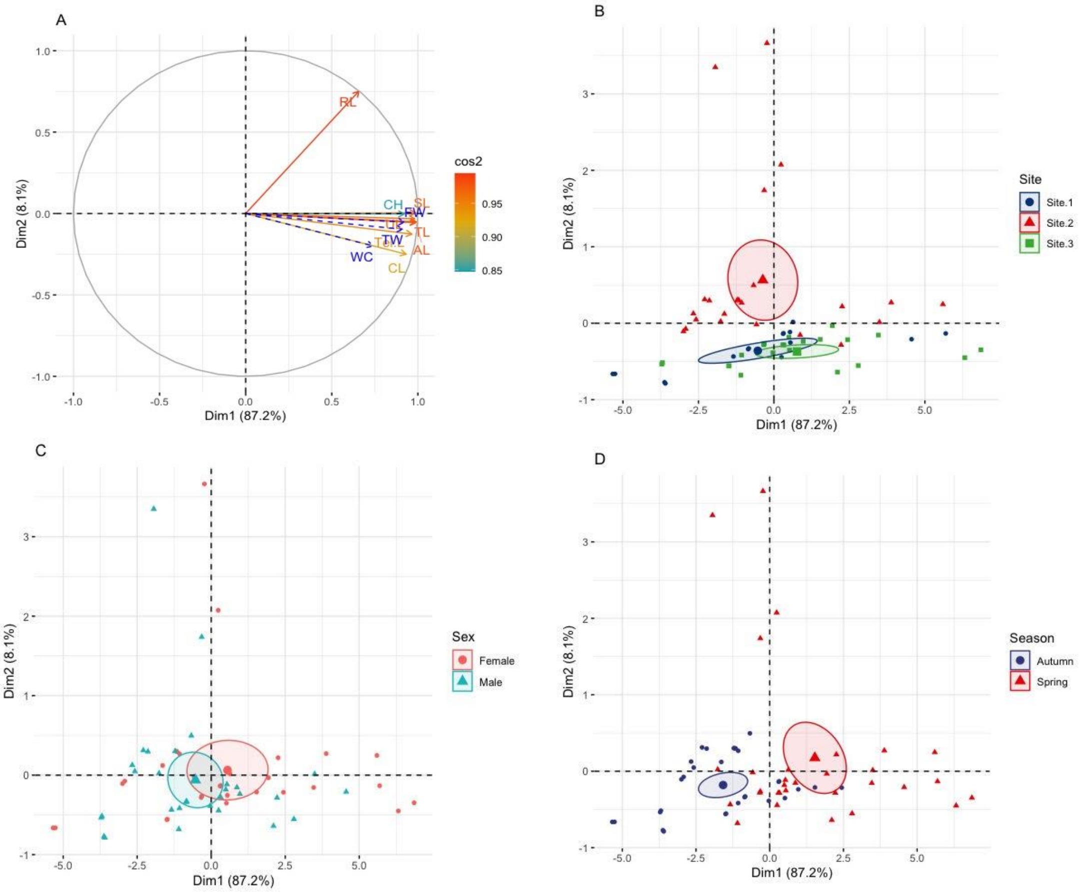

Figure 3. *Principal component analysis (PCA) carried out on the used morphometric characteristics of three populations in A. desmaresti, (A) correlation circle between the linear morphometric variables and the first two components; blue arrows represent the supplementary weight variables, (B) ordination plot according to the 'site' factor, (C) ordination plot according to the 'sex' factor and (D) ordination plot according to the 'season' factor*

The comparison between sites, by Kruskal-Wallis test, of the number of eggs carried by the gravid shrimps (*Fig. 4A*) revealed a significant difference ($p < 0.05$). Also, fecundity was recorded in a decreasing manner, respectively site 3> site 1> site 2. The comparison of the eggs' diameter in *A. desmaresti* (*Fig. 4B*) revealed a significant difference ($p < 0.05$) between site 2 compared to site 1 and 3. Thus, the decreasing order of egg's diameter is as follow: site 2> site 3> site 1.

Regarding the fecundity parameters and eggs diameter the correlation analysis using Spearman's correlation, showed that in the site 1, fecundity was weakly correlated with TL (r = 0.28), and with diameter (r = -0.23), and the no correlation was found between egg diameter and TL (*Fig. 5A*). In site 2, in addition the statistical analysis showed a very weak correlation between fecundity and TL (r = -0.03), egg diameter (r = - 0.15), while the correlation between egg diameter and TL was also very weak (r = 0.02)

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(*Fig. 5B*). For site 3, a positive correlation (*r* = 0.43) was found between fecundity and TL, and another negative correlation (*r*= -0.38) was also registered between fecundity and egg diameter; but between egg diameter and TL the correlation was also very weak (*r*= -0.05) (*Fig. 5 C*). The vitelline contents were compared, in ovigerous shrimp, where the Kruskal-Wallis test revealed significant differences (p <0.05) between site 3 compared to site 1 and 2 (*Fig. 6*).

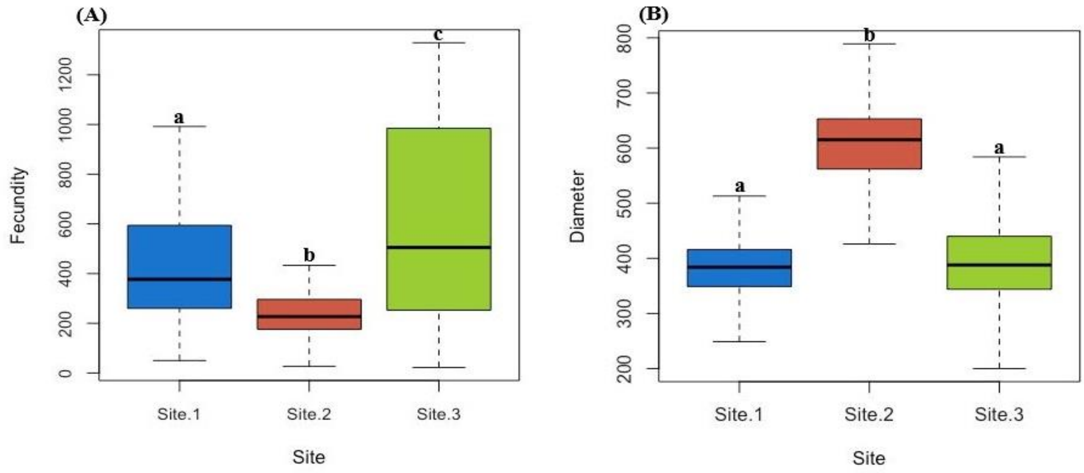

Figure 4. *Variations in fecundity (A) and egg diameter (B) of ovigerous females of A. desmaresti fished during spring 2021. Means followed by the same letter are not significantly different from each other at p > 0.05*

Responses of biochemical environmental biomarkers

The second objective of this work is to explain the seasonal biochemical responses of some biomarkers to environmental stress. So, the specific activity of AChE and GST, and the rate of MTs were determined in *A. desmaresti* tissues during spring and autumn in 2020. The specific activity of AChE showed a maximum AChE activity in individuals from the sites 2 and 3 as well as a minimal activity in the individuals from site 1 in spring. In autumn, a significant decrease in AChE activity at site 1 compared to sites 2 and 3. Comparison of AChE activity by the Kruskal-Wallis test between the studied sites revealed significant difference (p <0.05) between the site 1 compared to sites 2 and 3 during both seasons (*Fig. 7A*). GST activity values show a maximum in individuals from the sites 2 and 3 during the spring. A decrease in the GST activity during autumn is also observed within the three studied sites, where the induction is observed at sites 1 and 3 compared to site 2. Comparison of GST activity by the Kruskal-Wallis test between the study sites revealed significant difference (p <0.05) in autumn but no significant difference is recorded in spring (*Fig. 7B*). The levels of MTs are determined seasonally at the head of *A. desmaresti*. The results obtained reveal a maximum rate of MTs in individuals from the site 2 during the spring followed by site 1 and site 3. During the autumn, the highest MTs rate is observed at site 3 followed by site 1 and site 2. A decrease in MTs rates is observed at site 2 and site 1 during the autumn compared to the spring and a slight increase is noted at site 3 in the autumn. Comparison of MTs rates by the Kruskal-Wallis test between the three study sites revealed no significant difference (p> 0.05) during the spring and a very significant difference (p <0.05) between the study sites in autumn (*Fig. 7C*).

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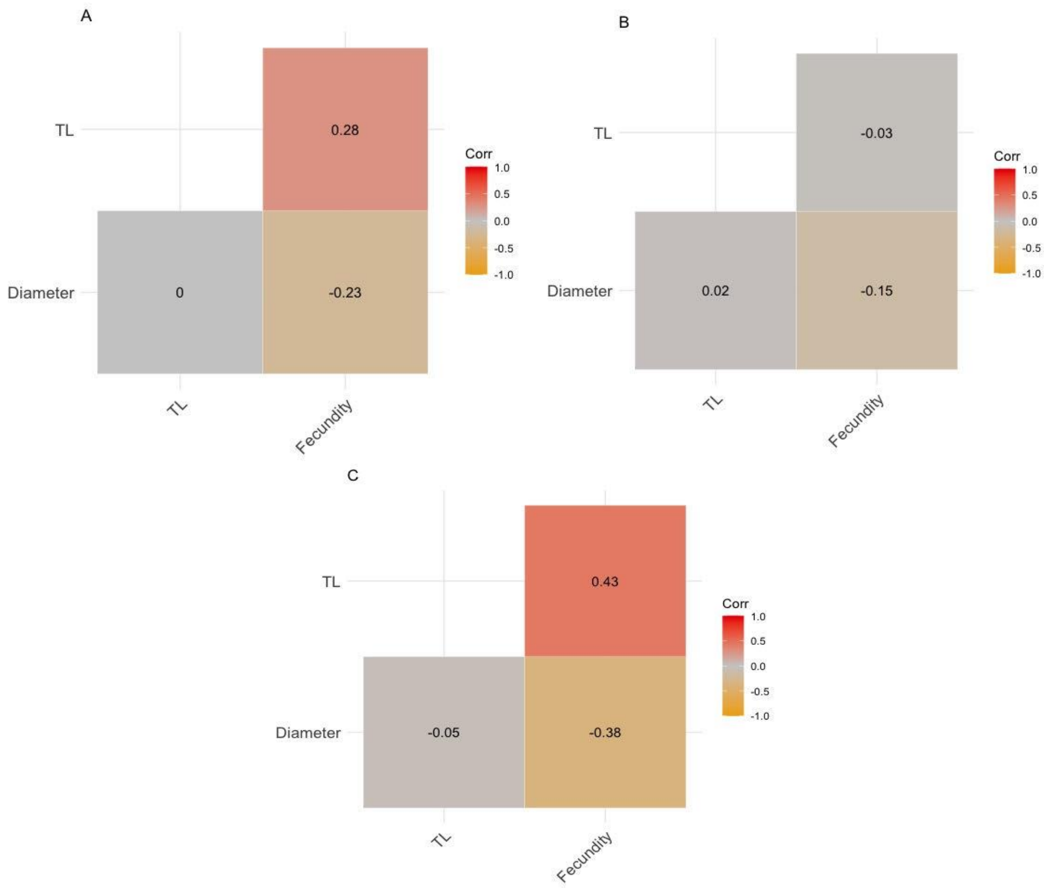

Figure 5. *Spearman's nonparametric correlation applied to the reproductive parameters of ovigerous females in A. desmaresti fished during spring 2021 in: El Battah (A), Oubeira (B) and Tonga (C)*

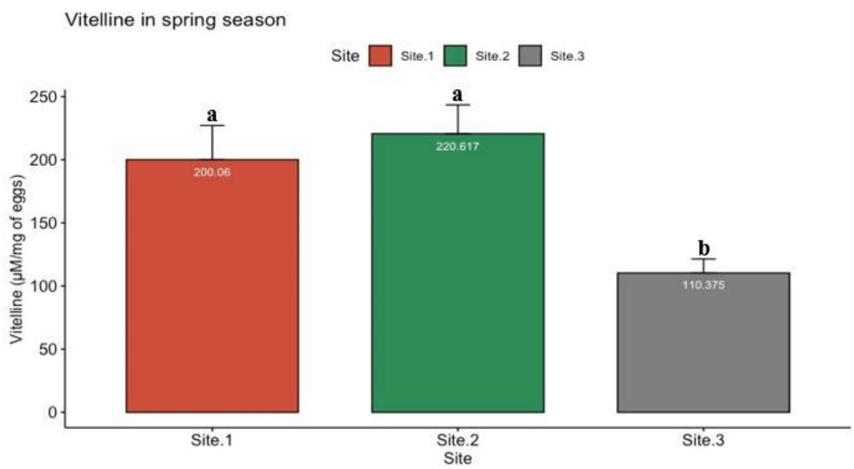

Figure 6. *Vitelline contents in eggs of ovigerous females of A. desmaresti fished during spring 2021. (m ± SE, n = 5; for each month of the season at each site, means values followed by the same letter are not significantly different from each other at p > 0.05*

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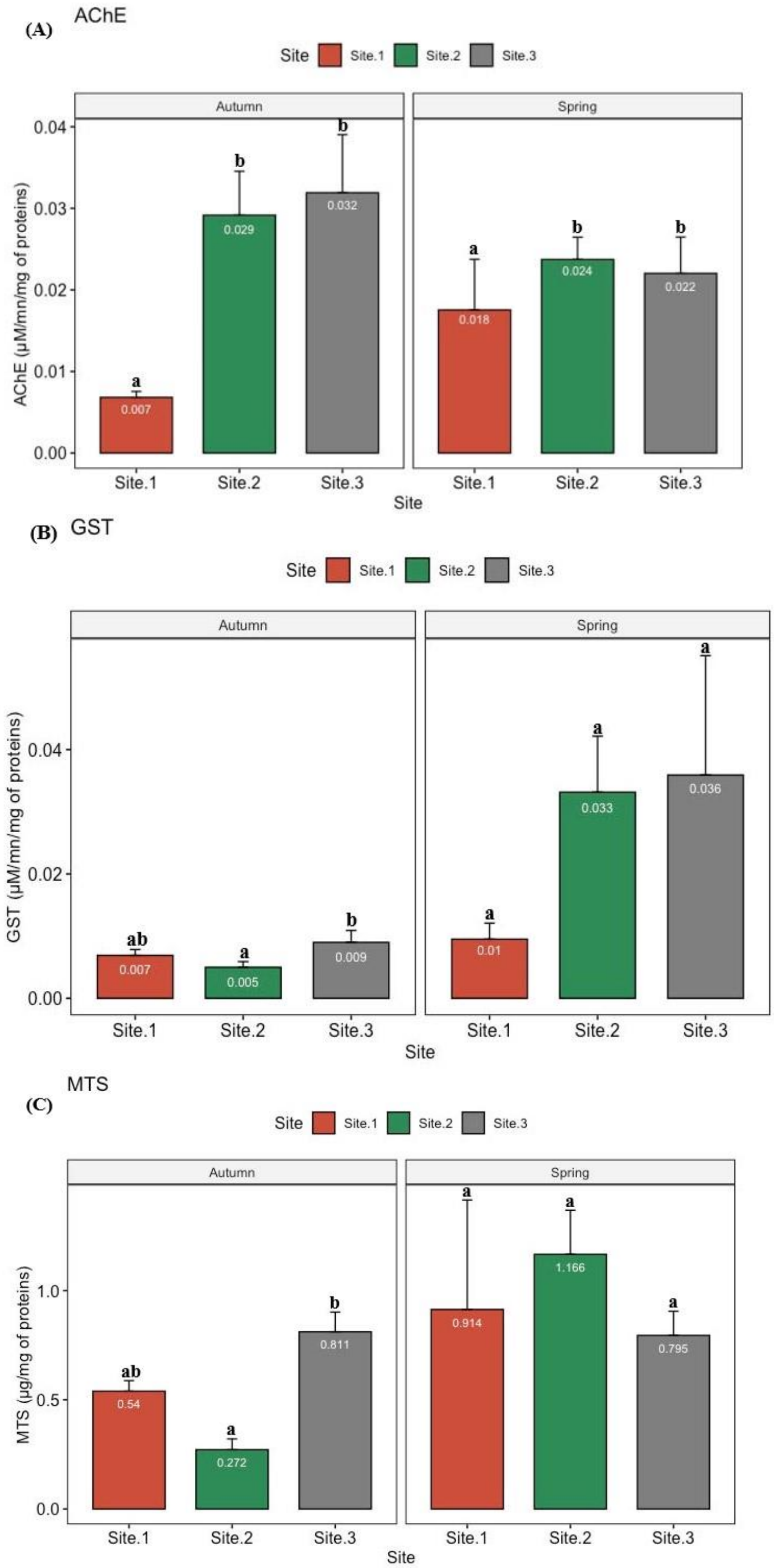

Figure 7. *Seasonal variations in specific acetylcholinesterase (AChE) (A), glutathione S-transferase (GST) activity (B), and MTs levels (C) measured in A. desmaresti individuals collected from the studied sites during the spring and autumn of the year 2020. (m ± SE, n = 5; for each month of the season at each site, means values followed by the same letter are not significantly different from each other at p > 0.05*

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Discussion

The physicochemical parameters play an essential role in the growth of biota in an aquatic ecosystem (Murugan et al., 2020). Temperature influences all physiological processes that occur in organisms, being particularly determinant for ectothermic animals (Sampaio and Rosa, 2019). Similarly, for pH salinity and dissolved oxygen (Sampaio and Rosa, 2019). The physicochemical characteristics of water from the different studied sites show a similarity between sites 2 and 3 during 2019-2020 period, with a peak of temperature (site 1, autumn), pH (site 2, autumn) and salinity (site 1, spring) in 2020. The dissolved oxygen peak was recorded at site 3 in fall 2019. So, an increase in temperature was recorded in 2020 compared to 2019. This is probably due to global warming and drought episodes that the world experienced during the year 2020 (World Meteorological Organization (WMO), April 2021), which could influence shrimp populations.

Crustaceans are recognized as good biological models for numerous ecotoxicological studies such as *Penaeus kerathurus* (Morsli and Soltani, 2003; Morsli et al., 2015), *Palaemon adspersus* (Benradia et al., 2016; Lechekhab and Soltani, 2018; Berghiche et al., 2018), *Artemia franciscana* (Varó et al., 2019), *Palaemon serratus* (González-Ortegón et al., 2015) and *Macrobrachium potiuna* (De Melo et al., 2020) or studies on seasonal growth in different species of Palaemonidae such as *Palaemon gravieri* (Kim, 2005), *Palaemon adspersus* (Manent and Abella-Gutierrez, 2006) and *Palemonetes antennarius* (Anastasiadou et al., 2009). This justifies the use of *A. desmaresti* in our study as a model for assessment of aquatic ecosystems health with morphometric, reproductive and biomarker approaches.

Measurements of length and weight of aquatic species are used to quantitatively assess species growth patterns, it is also, a biological response used as an indicator reflecting the effect of stress at the individual or population level (Khalil et al., 2021). So, the present study attempted to test the hypothesis that populations of *A. desmaresti* occupying three different habitats (fresh and brackish water) could show morphological and reproductive differentiation; and to screen a possible effect of pollution on the growth and reproduction of this shrimp species. The weight and linear growth of the *A. desmaresti* revealed a significant positive correlation between the different morphometric parameters studied in three habitats during two years. Similarly, Konan et al. (2017) investigated the morphometric characteristics of the shrimp *Macrobrachium macrobrachion* in Ivory Coast (Africa) where all the relationships between the variables considered (length-length and weight-length relationships) were significantly linear. The same observation was made by Hayd and Anger (2013) on *Macrobrachium amazonicum* from the Pantanal in Brazil.

The correlation between the biometric parameters in the Algerian populations of *A. desmaresti* showed that this species develops (growth process) in the same way in their habitats despite the difference between brackish (site 1) and freshwater (site 2, 3). Which refers to the euryoecological and eurythermal characteristics of the shrimp, as it is abundant in several freshwater or brackish lake. It tolerates very large variations of salinity and temperature with a hyper-iso-osmotic regulation (Dhaouadi et al., 2006). The results clearly demonstrated an abnormal growth of the rostrum in shrimps fished at site 2 (freshwater). This phenomenon could be a morphological deformation because it is common in crustaceans’ fauna. Actually, the condition of Oubeira Lake has deteriorated over time, and is characterized by high concentrations of iron, zinc, manganese, chromium and lead (Bendjama et al., 2014). Thus, the malformations of the

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DOI: http://dx.doi.org/10.15666/aeer/2006_45794597

rostrum of *A. desmaresti* could be due to the bioaccumulation of these substances, as the causal factor of morphological alterations in crustaceans are pollutants. Also, a study conducted by Beguer et al. (2008) in the Gironde estuary (French), showed exoskeletal deformity is a major phenomenon that has affected some shrimp of the Palaemon genus (*Palaemon longirostris*, *Palaemon macrodactylus* and *Palaemon serratus*) for over 15 years, and up to 40% of adult shrimps were suffering from deformities. Morphological anomalies have been reported in crabs, crayfish and shrimps (Aguirre and Hendrickx, 2005). Different anomalies have been reported, affecting sexual appendices (Rodriguez and Campos, 2000), chela duplication forked telsons, rostrum size, bifurcation, curvature, the number of teeth or absence of teeth of the rostrum and asymmetry reversal (Aguirre and Hendrickx, 2005).

Our results revealed a lower abundance of male individuals of *A. desmaresti* than females confirming works made in other geographical localities with the same species like Minho River in Portugal (Fidalgo et al., 2015), Meuse River in Belgium (Meurisse-Génin et al., 1985) and in three Tunisian reservoirs: Sidi Salem, Lebna and Sidi Saad (Dhaouadi-Hassen et al., 2006). The abundance of *A. desmaresti* is also higher in spring than in autumn. A result similar to that of Overko et al. (1983) where they revealed that the distribution of shrimp varies according to season, depth and regions and that in spring the average catch per trawling hour was twice as high as in autumn. Also, the abundance of *A. desmaresti* in 2019 is much higher than that recorded in 2020; this is likely due to global warming and the dry spells the world experienced that year (World Meteorological Organization – WMO –, April 2021). The abundance of *A. desmaresti* was greater in sites 2 and 3 than in site 1, possibly due to the water currents that the Mafragh (El Battah Estuary) is constantly subjected. In contrast to sites 2 and 3, where shrimp populations living in the estuaries often migrate in quantity and downstream.

Bamber and Henderson (1994) reported that the majority of estuarine resident Caridae move seaward to release their eggs away from the shore (to avoid storm damage) and because phytoplankton production is greater under marine conditions, which allows for good feeding. This is why we recorded a large number of individuals at site 2 and site 3 compared to site 1.

Our results also showed that the highest fecundity was recorded at site 3 (618.66 eggs), then at site 1 (340.67 eggs) and finally the lowest fecundity was observed at site 2 (229.87 eggs). These values are in perfect agreement with those of Dhaouadi-Hassen et al. (2006) who have measured the fecundity in *A. desmaresti* females in several Tunisian barrages like Sidi Salem (313 eggs) Sidi Saâd (591 eggs) or Lebna (459 eggs). Our results are also in agreement with those of Schoolmann et al, (2015) who revealed that fecundity in *A. desmaresti* varies between 137 and 1380 eggs with an average number of 666 eggs per female per laying. In contrast, the diameter of the eggs is larger at site 2 (599.50 µm) than at site 3 (421.08 µm) and at site 1 (383.38 µm). However, Somers (1991) reported that the number of eggs depends less and less on the size of the ovigerous females which is surprising in crustaceans and could be related to the presence of morphological abnormalities found in shrimps, especially large ones. Environmental factors may also influence the absolute number of eggs carried by females.

A negative correlation was noted between egg diameter and fecundity at site 1 (r = -0.23), site 2 (r=-0.15) and site 3 (r=-0.38). Our results are also in agreement with those performed on long-term monitoring (1979-2007) of the *Palaemon longirostris* from the Gironde estuary, where they revealed that egg volume is negatively correlated

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with egg number. This increased variability could be a response to excessive environmental or anthropogenic pressure (pollution). Decapod egg volume is related to the chemical composition of the water, in particular salinity, but also temperature (Wehrtmann and López, 2003). Thus, egg volume decreased with increasing both temperature and salinity, which is in agreement to our study in which the largest diameter was found at site 2 (freshwater) with lower temperature compared to site 1 and site 3. Then we have the egg diameters from site 3 where both salinity and temperature were more important than site 2. Finally, the smallest diameter was recorded at site 1, where temperature and salinity were more important than Lakes (sites 2, 3).

The reserves of vitelline (Vn), a high-density lipoglycoprotein associated with carotenoid pigments available to the larvae after hatching allow them to live without feeding until the first molt. Yolk is a biomarker of defense against toxic exposure (Lechenault, 1968), so in order to analyze the likely adverse effect of pollution on reproduction, yolk levels were determined in adult females. In our study, Vn levels are high at site 2; this is probably due to the large diameter of the shrimps' eggs. Although egg diameter at site 3 is slightly larger than at site 1, yolk content site 1 was significantly higher than site 3, probably due to the metal pollution, high levels of iron, copper, zinc, and lead (Bendjama et al., 2014), these substances have probably disrupted the reproduction of *A. desmaresti* at this site.

Recently, the great focus on the health of aquatic ecosystems has led to an increase in the use of biomarkers. The advantage of this approach was that the biomarker levels recorded were manifested at the organism level under the influence of water pollution. The AChE is a key enzyme in the nervous system, catalyzing the hydrolysis of the neurotransmitter acetylcholine (Lionetto et al., 2019). Thus, comparison of AChE activity in *A. desmaresti* was similar in our study sites 1, 2 and 3 during the autumn and spring. The natural AChE activity is not directly related to the age, sex or the reproductive period of the organism and previous work recognizes inhibition of AChE activity as an indicator of exposure to pollutants such as organophosphate and carbamate pesticides, heavy metals, detergents, algal toxins (Grintzalis et al., 2012). The significant difference between site 1 compared to sites 2 and 3 was probably due to reject of neurotoxic substances compared to the two lakes Oubeira and Tonga. GSTs are a large complex family of enzymes localized in the cytosol and endoplasmic reticulum that acts as important regulators in many of the metabolic steps of redox and detoxification (Painefilú et al., 2020). Data on GST activity revealed a significant difference between the sites in autumn, while no difference was recorded in the spring; when the enzymatic activity was maximal. The induction of GST activity in *A. desmaresti* fished from site 3 is most probably related to the metal pollution (Bendjama et al., 2014). A significant induction in GST and a significant inhibition in AChE activities in individuals *P. adspersus* from the sites in the output of El Mellah River and in the output of R'kibet River (near to anthropogenic activities) compared to the site in the channel of the Mellah Lagoon (as reference site) are related to their level of pollution (Benradia et al., 2016). Denna et al. (2022) recorded a significant differences in GST and AChE activities in *Gambusia affinis* at three sites in northeastern Algeria most polluted site of Sidi Brahim, followed by El Karma and finally the Messida River. MTs are the proteins most susceptible to metal contamination in many marine organisms (Choi et al., 2008). They are involved in the homeostasis of essential metals such as copper and zinc, but also play an important role in the detoxification of nonessential metals (Chandurvelan et al., 2015). In our study, the MTs quantification

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revealed high levels in the spring with no significant differences between sites, but a significant difference was observed in the autumn with a high level at site 3. Results are in agreement with those of Bendjama et al. (2014) which revealed the presence of high levels of iron, copper, zinc and lead at site 3. Benradia et al. (2016) showed a significant induction in GST and also a significant inhibition in AChE activities in individuals from the output of El Mellah River and the output of R'kibet River compared to the channel of the Mellah Lagoon. Mts induction is considered a good biomarker of exposure to trace metal (TM) and is commonly used in environmental biomonitoring programs (Falfushynska et al., 2009). However, the accumulation of MTs in tissues could also be due to oxidative stress independently of the presence of TMs in the environment. So, the reduction in AChE activity in shrimp tissue is most probably related to the distribution of pollutants in neural tissue, while GST activity confirmed a generalized antioxidant stress response. The high MTs levels indicate a metal pollution. The quality and availability of water resources are undoubtedly one of our main challenges. Its protection requires better management of pollutants, mainly the pollution of beaches, lakes, rivers and lagoons by human activities.

Conclusion

The positive correlation between the biometric parameters of the shrimp *A. desmaresti* refers to the euryoecological and eurythermal characteristics. A deformation of the rostrum of the shrimps at site 2, probably caused by heavy metals. Population abundance of *A. desmaresti* was higher in 2019 compared to 2020, due to drought periods, and the abundance was higher in sites 2 and 3 then in site 1. The site 1 is constantly subject to water currents, as shrimp in the Mafragh Estuary quantitatively migrate downstream. A negative correlation between egg diameter and fecundity and a low correlation between total length and fecundity were recorded in *A. desmaresti* populations, with the highest fecundity at sites 3, 1, and 2, and the largest diameter at sites 2, 3, and 1. Biochemical analyses revealed a significant difference in GST activity, MTs and vitelline levels where the contamination was recorded at site 3. AChE, GST and MTs were valuable biomarkers for assessing the response of shrimps to pollutant exposure, which represents a stress alert for shrimp, and suggests that it would be useful to identify the nature of the pollutants.

In the future and in order to give more morphometric characterization of the shrimps' populations; the morphological structure should be assessed by a geometric morphometric method based on the landmarks configuration which are powerful to appraise shape and size variations among the Algerian populations of *A. desmaresti*.

Acknowledgements. This research was supported by the Algerian Fund for Scientific Research of Algeria (Laboratory Applied Animal Biology to Pr. N. Soltani) and by the Ministry of Higher Education and Scientific Research of Algeria (PRFU project: D01N01UN230120120190008 to Pr. S. Chouahda).

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ELECTRONIC APPENDICES

Table A1. *Seasonal variations of biometric parameters at A. desmaresti shrimp during 2019 with the (Mean/Minimum value/Maximum value) in the three study sites*

Table A2. *Seasonal variations of biometric parameters at A. desmaresti shrimp during 2020 with the (Mean/Minimum value/Maximum value) in the three study sites*

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