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Radia Mebarki
Laboratory of Applied Animal Biology Department of Biology, Faculty of Sciences, Badji Mokhtar University 23000-Annaba, Algeria.

Mohamed El Hedi Khebbeb
Laboratory of Applied Animal Biology Department of Biology, Faculty of Sciences, Badji Mokhtar University 23000-Annaba, Algeria.

Noureddine Soltani
Laboratory of Applied Animal Biology Department of Biology, Faculty of Sciences, Badji Mokhtar University 23000-Annaba, Algeria.

Correspondence
Mohamed El Hedi Khebbeb
Laboratory of Applied Animal Biology Department of Biology, Faculty of Sciences, Badji Mokhtar University 23000-Annaba, Algeria.

Biomonitoring of El Mellah Lagoon (Northeast, Algeria): Seasonal Variation of Biomarkers in *Cerastoderma glaucum* (Mollusc, Bivalvia)

Radia Mebarki, Mohamed El Hedi Khebbeb, Noureddine Soltani

Abstract
The present study aimed to measure the seasonal activity of glutathione S-transferase (GST) and acetylcholinesterase (AChE) biomarkers of oxidative stress and neurotoxicity in a mollusc species *Cerastoderma glaucum* Bruguiere, 1789 (Mollusca, Bivalvia). The experiment was done during one year (April 2010 to March 2011) in three sites located in El Mellah lagoon. Environmental parameters (temperature, dissolved oxygen and salinity) were also monitored. Results demonstrated that the physic-chemical data put in evidence of the temporal fluctuations without station effects. *C. glaucum* showed differential biomarker response according to abiotic factors and of the anthropogenic pressure of pollutants that affect the lagoon. An increase in GST activities was usually observed in bivalves from the polluted sites 1 and 2 compared to the reference site 3 could be to reflects the induction of detoxification system. Moreover, a significant inhibition in AChE activities was recorded in bivalves from the polluted sites suggesting contamination by neurotoxic pollutants such as heavy metals. Overally, obtained data indicate that *C. glaucum* constitutes a useful tool as sentinel organism for biomonitoring of aquatic pollution.

Keywords: Biomonitoring; Cerastoderma glaucum; Algeria; Lagoon; El Mellah; Biomarkers; GST; AChE.

1. Introduction

The pollution of aquatic ecosystems by several pollutants is an important environmental problem [1, 2]. During the last decade, various studies have shown that hydrocarbons [3], metals [4, 5], [6, 7, 8], organophosphorus compounds (PCBs), pesticides and herbicides considerably contaminate different compartments of industrialized coastal regions. The absorption of some pollutants takes place in Humans mostly via the intake of contaminated food. Molluscs are present in our diet, they are great bioaccumulators even if they originate from sites in which the levels of such contaminants are considered low [9] and could be considered ‘potentially’ dangerous for consumers [10].

Pollution of aquatic environment can be estimated in water, sediment and also in marine organisms [11]. According to [12] a bioindicator is an organism or a set of organisms that allows, by reference to biochemical, cytological, physiological, ecological or ethological variables, in a practical and safe way, to characterize the status of an ecosystem or an ecocomplex and to highlight as early as possible their changes, natural or caused. Species frequently used as indicators are bivalve molluscs [4, 13, 14, 15] as *Cerastoderma glaucum* Bruguiere, 1789. This specie is an excellent indicator of contamination because of its strong ability to bioconcentrate xenobiotics [16, 17, 18].

The environmental risk assessment involves the use of biomarkers designed to highlight an early stage of pollution [20, 21]. Many biochemical and cellular biomarkers have been studied in aquatic organisms, and particularly in fish and bivalve molluscs [21, 22]. Glutathione S-transferase are a multiple-enzyme family involved in phase II detoxification processes [23] and are used as biomarkers of several groups of pollutants including metals and organophosphorus [24, 25]. Acetylcholinesterases play an important role in the functioning of the neuromuscular system by preventing continuous muscular contraction [26]. AChE activity has been proposed as a biomarker of exposure to several chemicals such as organophosphorus compounds [27], and also by other contaminants such as metals, synthetic detergents, some components of fuel oils and algal toxins [29, 39].

The aim of this study was to assess monthly pollution variation during one year by evaluating Activities of two biomarker, GST and AChE, in *C. glaucum* fished in an El Mellah Mediterranean Coastal lagoon (Northeast Algeria).

2. Materials and Methods

2.1 Study Area

Cockles *Cerastoderma glaucum* Bruguiere, 1789 (Mollusca, Bivalvia) were collected from April 2010 to March 2011 at El Mellah Lagoon located in the extreme eastern Algeria near the Tunisian-Algerian border in the region of El Kala (36 ° 53 '50'N, 8 ° 19' 30 'E) (Fig. 1), it appears as an elongated ovoid bowl in a dense forest cover naturel site. It is connected to the sea by a channel 900 meters long and 1 to 10 meters wide. This lagoon is not only fueled by marine waters, which are partly responsible for its brackish nature, but also by the gentle waters of three rivers: R'kibet Northwest, and South El Mellah and Belaroug. Three sites were choosen for collecting cockles (Fig. 1). The sites 1 (36°54' N, 008°18' E) and 2 (36°52' N, 008°19' E) were known to be more polluted than the site 3 (36°53' N, 008°20' E) because of their location near rivers, which contains several pollutants, originate from urbanism factory rejects.

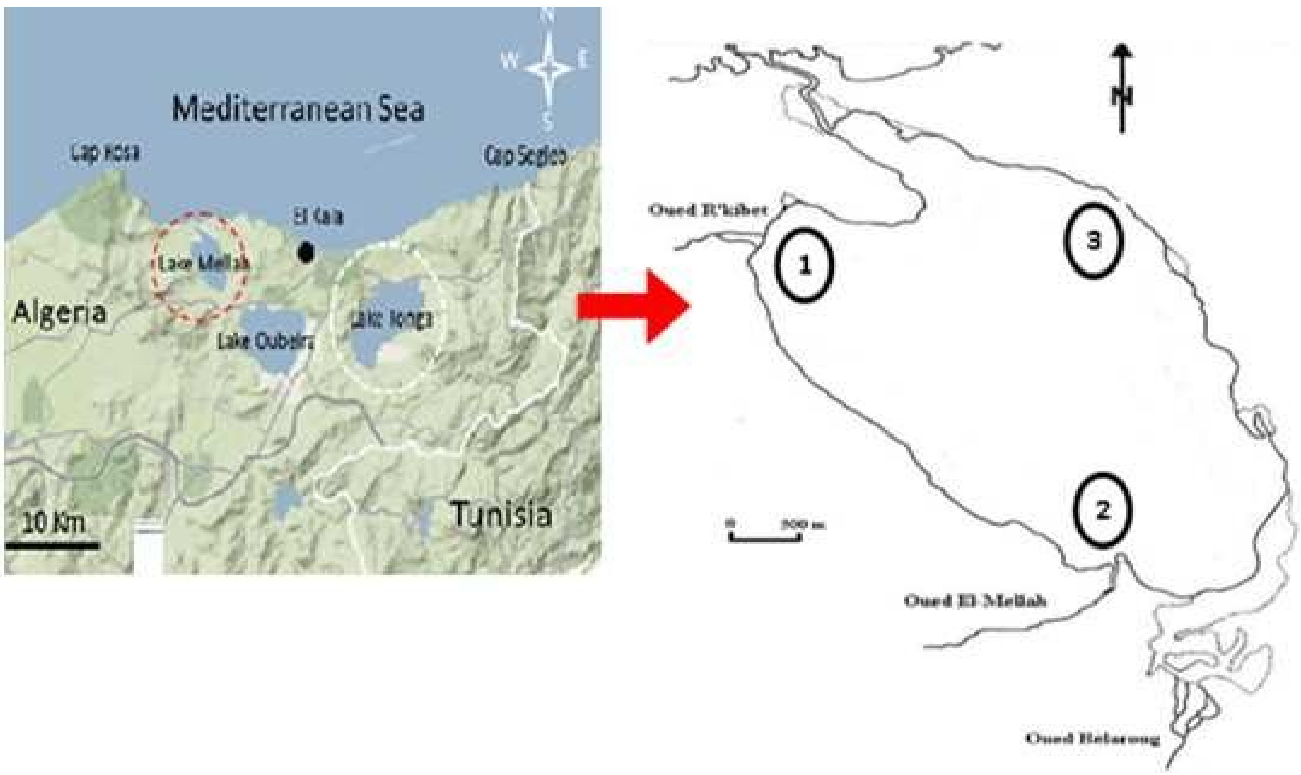

Fig 1: Location of sampling sites in El Mellah Lagoon (Northeast Algeria).

2.2 Determination of Physico-Chemical Parameters of Water

Physico-chemical parameters were measured monthly from April 2010 to March 2011. Temperature, dissolved oxygen and salinity of water of the three sites were measured in duplicate *in situ* using a multi-parameter WTW type 2F30104211 (Multi 340 I/Set).

2.3. Collection and preparation of samples

C. glaucum of similar size (26 ± 1 mm) were monthly collected by hand between April 2010 and March 2011 from different sites and were immediately transferred to our laboratory and dissected. The digestive gland and gills were used as biological material for the quantification of GST and AChE activities, respectively. The digestive gland was kept in homogenization buffer (20 ml of Phosphate buffer 0.1M pH= 6, 1.71g sucrose) using ultrasound. The homogenate was then centrifuged at 40000 rpm for 30 minutes. The recovered supernatant was used for the determination of GST and protein testing. Gills were homogenized for a few seconds in 1ml of detergent solution (38.03 mg Tris-ethylene glycolbeta-aminoethyl ether N N N 'N' or EGTA, 1 mL triton X 100%, 5.845 g NaCl, 80 ml 10 mM Tris buffer) and centrifuged at 9000 rpm for 5 minutes. The recovered supernatant was used for determination of AChE activity and protein concentration.

2.4. Glutathione S-Transferase Activity

GST activity was quantified according to the colorimetric method [30] and expressed as µM per min per mg of proteins, of providing a substrate for enzyme (usually 1chloro2, 4 dinitrobenzene CDNB which reacted readily with many form of GST) and glutathione. The catalyzed reaction of conjugation of this two products lead to the formation of a new molecule that absorbed light at 340nm. Activity was expressed as µmol/min/mg protein.

2.5. Acetylcholinesterase Activity

Determination of AChE activity was performed using a method described by [31] with the use of acetylthiocholine (ASCh) as substrate. The activity rate was measured as change in absorbance/min at 412 nm (extinction coefficient 13.6 Mm.cm). Activity was expressed as nmol/min/mg protein.

2.6. Protein Quantification

The proteins were quantified using bleu brilliant of Coomassie (G250, Merck) as reagent and bovine serum albumin (BSA, Sigma) as standard protein [32]. The absorbance was read at a wavelength of 595 nm.

2.7. Statistical Tests

Data are expressed as mean ± standard deviation (SD). All statistical calculations were performed with the MINITAB

Software (Version 16, Penn State College, PA, USA). Data from physico-chemical parameters water and enzyme activities (GST, AChE) were tested using two-way analysis of variance (ANOVA). Differences between sites were determined by Tukey's test. A significant difference was assumed when p<0.05.

3. Results

3.1. Physico-Chemical Characteristics of Water from Different Sites

Physical parameters (temperature, dissolved O_2 and salinity) monthly measured in water were presented in table 1. Obtained results showed seasonal variations in the three sampling sites along a year and no significant difference was observed between monthly data between the different sites of each studied parameters (Table 2). The lowest temperature was recorded in January, for against the highest was registered in August with a maximum values of 30.5 °C at sites 1 and 2. The values recorded for the dissolved oxygen in lagoon water usually exceeded 4 mg/l at the three sites of studies. Dissolved oxygen concentrations from the lagoon, above 8 mg/l were recorded from October 2010 to May 2011, with a maximum of 14.7 mg/l in December at the site 3; however, during summer concentrations of O_2 in the water were lower (close to 4.2 mg/l at site 2 in August).

Salinity records showed the highest values in summer and autumn period, with a maximum value of 27.5 psu at the sites 1 and 2 during the month of August. However, in winter, salinity reached their minimum value 15.8 psu in February at the three studied sites.

Table 1: Temperature (°C), dissolved oxygen (mg/L) and salinity (psu) monthly variation during one year in the three studied sites.

Parameters / Months	Temperature (°C)			dissolved oxygen (mg/l)			Salinity (psu)		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
April	17.8	18.1	18.0	8.1	8.1	8.7	20.3	19.3	21.4
May	21.1	22.3	21.0	7.7	7.5	7.9	20.4	20.5	21.3
June	25.9	26.1	25.6	7.4	7.3	7.8	23.5	23.4	23.1
July	28.4	28.5	28.2	6.3	6.7	7.1	25.15	25.2	25.0
Aug	30.4	30.5	30.5	4.5	4.2	5.3	26.30	26.2	27.3
Sept	25.6	25.4	25.5	6.3	6.9	7.1	27.50	27.15	27.5
Oct	17.3	17.4	17.4	8.3	8.6	8.4	25.25	25.29	25.25
Nov	15.9	15.7	15.7	12.2	12.1	12.7	26.90	24.14	25.10
Dec	12.7	12.5	12.8	13.9	13.7	14.7	20.40	20.35	20.37
Jan	12.2	12.2	11.8	11.9	11.4	11.4	17.5	17.4	17.5
Feb	12.1	11.9	12.1	9.3	9.5	9.7	15.8	15.8	15.8
March	15.1	15.1	15.0	9.7	9.1	9.6	15.9	15.9	15.9

Table 2: Two-way ANOVA (site, month) on physico-chemical water parameters from the El Mellah lagoon (***: significant).

Physical parameters	P (Site)	P (Month)	P (Site × Month)
Temperature (°C)	0.988	0.001***	1.000
Salinity (psu)	0.853	0.001***	1.000
Dissolved oxygen (mg/L)	0.784	0.001***	1.000

3.2. Glutathion S-Transferase Activity

Monitoring of the variation in the content of GST (m ± s) (µM /min /mg protein) in the *C. glaucum* [16] cockle at the three sites revealed the existence of monthly significant fluctuations (Fig. 2). GST content increased and the maximum values was recorded in July at the site 2 with a value of 58.62 ± 0.547 µM / min / mg, and in August for the sites 1 and 3 with the following values 52.76 ± 3.325 and 45.10 ± 3.608 µM/min/mg, respectively. The lowest values was recorded in December in all sites. In addition, GST activity in digestive glands from sites 1 and 2 were significantly higher than those measured in individuals from site 3. Significant effects (P< 0.001) of site (F=369.14; df= 2. 108) and months (F= 254.72; df=11. 108) were revealed by two-way ANOVA test (Table 3).

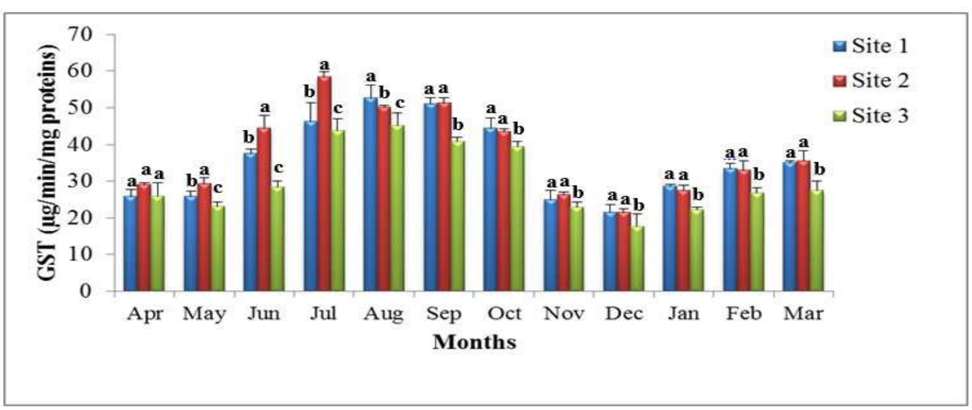

Fig 2: Monthly variation of glutathione -S-transferase (GST) activity (µM/min/mg protein) in digestive glands of *C. glaucum* collected from different sites at El Mellah Lagoon (April 2010 to March 2011). (m± SD ; n= 6 ; for each month, mean values followed by different letters are significantly different p>0.05).

Table 3: Two-way ANOVA (site, month) on GST activity data in C. glaucum digestive glands.

Sources of variation	DF	SS	MS	Fobs	P
Site	2	3526.4	1763.21	369.14	0.001
Month	11	13383.7	1216.70	254.72	0.001
Interaction Site/Month	22	1191.3	54.15	11.34	0.001
Residual error	108	515.9	4,78	-	-
Total	143	18617.3	-	-	-

DF: Degrees of freedom **SS:** Sum of squares **MS:** Mean squares

Acetylcholinesterase Activity

AChE activity was evaluated on mollusc gills fished in the different sites (Fig. 3). Obtained results showed significant fluctuations of this activity along the year. AChE activity from site 3 was higher than those recorded in sites 1 and 2. The lowest values were observed in August at the three sites of study, with values of 16.78±1.014 nmol/min/mg protein for site 1, 12.27±1.801 nmol/min/mg protein to level the site 2, and 24.32±1.629 nmol/min/mg protein for the site 3. The maximum values were observed in the spring. Results were confirmed by the two-way ANOVA since a significant ($P< 0.001$) effect of both site ($F= 583.36$; df= 2. 108) and month ($F= 542.57$; df= 11.108) was noted (Table 4).

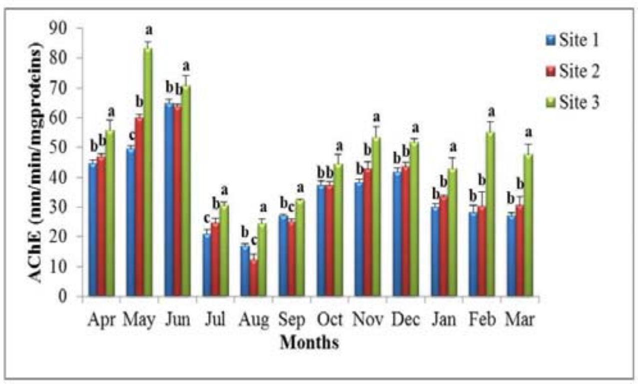

Fig 3: Monthly variation of acetylcholinesterase activity nM/min/mg protein) in gills of *C. glaucum* collected from different sites at El Mellah Lagoon (April 2010 to March 2011). (m± SD; n= 6; for each month, mean values followed by different letters are significantly different p>0.05).

Table 4: Two-way ANOVA (site, month) on AChE activity data in C. glaucum gills.

Sources of variation	DF	CS	MC	Fobs	P
Site	2	5209.4	2604.68	490.43	0.001
Month	11	27566.1	2506.01	471.85	0.001
Interaction Site/Month	22	2111.1	95.96	18.07	0.001
Residual error	108	573.6	5.31	-	-
Total	143	35460.1	-	-	-

4. Discussion

The physical parameters of the water from El Mellah lagoon during an annual cycle (April 2010-March 2011) were globally similar in the three study sites. There was a seasonal fluctuation related to local climatic conditions and especially with the air temperature; this was attributable to the shallow depth of the water column (≤40 cm at the studied sites). The lowest temperature values were observed during the period of October 2010 to March 2011 with a minimum (11.8 °C) recorded in January at site 3, linked to high oxygen content due to the temperature drop and the increase in the brewing water. As observed in O_2 content drop in the period spread out from April to September 2010, which would be linked not only to the sharp rise in temperature that limits the solubility of oxygen but also for the respiration of aquatic organisms living and hydrodynamic calm. Regarding salinity, low values were recorded during the three-winter month (January, February, and March); this was due to the high water dilution in the lagoon by origin having heavy rainfall and low evaporation water. [33, 34] had reported similar observations on the physicochemical parameters of the El Mellah lagoon.

In this current study, evaluation of biomarkers (GST, AChE) revealed seasonal variations as also previously reported [35]. The maximum values of GST in *C. glaucum* digestive glands were recorded in the warmer months (June, July, August, and September) at the three sites; this was related to environmental factors such as temperature. Indeed, the temperature could influence the activity of enzyme systems by altering all biological and physiological functions of animals [36]. Other factors could influence the activity of GST such as the reproductive cycle [37]; age [38]. Inhibition of AChE activity was observed in the warm months (August). The same observations were noted [39] in *Mytilus galloprovincialis* in the lagoon of Bizerte (Tunisia) with increasing temperature. The activity of this enzyme could be modulated by natural factors such as seawater temperature, biotoxins or cyanobacteria and salinity [40, 41]. Environment contamination probably played a role, especially in sites 1 and 2 of lagoon compared to site 3. We found the highest levels of GST activity and the lower values of AChE activity in sites 1 and 2 compared with values measured in site 3. In both sites, 1 and 2 flow two rivers: R'kibet at the site 1 and El Mellah and Belaroug at the site 2. These rivers drain urban and agricultural discharges causing a contamination of sites 1 and 2. Moreover, a previous study on lagoon sediments of El Mellah showed a pollution of these two sites by heavy metals [42]. Rivers flowing into this part of the lagoon was contaminated by fecal germs [43].

In contrast, the site 3 was relatively less polluted. The low levels of GST activity and high levels of AChE as compared with the two other sites 1 and 2 confirmed this. It was considered as a reference site [44]. Our results were consistent with studies on *Donax trunculus* from the coast of Annaba (Algeria) [14, 45, 46]. They reported an induction in GST activities and an inhibition in AchE activity in the site of Sidi-Salem polluted by heavy metals compared to a less polluted site (El Battah). Similarly, an inhibition of AChE was observed in clams *Ruditapes philippinarum* exposed to neurotoxic pollutants present in the waters of agricultural land drainage [11]. And in *Donax trunculus* from a multi-contamination site by the intensive agricultural activities in comparison with the reference site from the Gulf of Tunis [47]. GST has a large response to several contaminants such as cadmium [48] and PAH [49].

5. Conclusion

It can be concluded that activities of GST and AChE were influenced by environmental factors (temperature, dissolved oxygen and salinity) this was confirmed by the seasonal variations of these enzymes during the study period. In addition, the sites 1 and 2 were more polluted compared to site 1. *C. glaucum* was useful as sentinel species to assess the environmental effects of pollution in this lagoon.

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